THE

AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

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The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

Public Relations Counsel, Glassner & Associates, Chicago, Illinois.

American Society of Medical Technologists

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The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 22 MAY-JUNE, 1956 NUMBER 3

FLAME PHOTOMETRY IN THE CLINICAL LABORATORY*

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Flame analysis was utilized as early as 1860 by Kirchoff and Bunsen so the qualitative value of emission spectra has been recognized for almost a century. Lockyer began quantitative spectrochemical analysis in 1873 but it was not until 1928 that the value of flame spectrophotometry was extensively investigated. At this time Lundegardh, using a high pressure air-acetylene flame, an atomizer, a quartz prism and photographic plates to record emission spectra, found about forty elements in solution were excited sufficiently by the low temperature flame to be measured. Direct reading machines were made possible when Jansen, Meyes and Richter in 1935 added a photocell for detection of radiation.[1]

In 1945 Barnes, Richardson, Berry and Hood demonstrated the simplicity and accuracy of a photometer employing a filter, a photocell and simple flame and spray equipment.[1] Berry, Chappell and Barnes went on to further improve the filter photometer by adding another filter and photocell, making it possible to employ the internal standard method, thereby eliminating many sources of error of the direct reading machine.[2]

Dilute solutions of the material to be analyzed are atomized and burned. Elements in these solutions are excited by the flame and emit characteristic spectra. With appropriate filters the emission of one element can be isolated and then focused upon a photocell which responds linearly to light energy directed upon it. Since light energy emitted is in direct proportion to the concentration of the element being detected, response of the photocell is also directly related to concentration, and unknowns may be determined by comparing their response to that of known standards.

* First Award, 1955, Scientific Products Foundation, Educational and Procedural Techniques Division.

The internal standard principle makes simultaneous use of two photocell-filter units, one for the detection of the unknown element's emission and one for that of the substance to be used as internal standard. Lithium is commonly chosen for the internal standard substance since its determination is seldom required in biological materials and because it emits only one line, 6708 Å, located conveniently between the analytical lines of sodium, 5890 Å, and potassium, 7690 Å.[3] Lithium is added to both unknown and standard solutions in amounts which will give an identical concentration of the internal standard in all solutions to be analyzed. On excitation of the unknown sample in the flame, emissions from the lithium and from the unknown element being measured are selected by filters and focused upon their appropriate photocells. Currents generated by both photocells oppose each other through the galvanometer; a potentiometer is adjusted until no current flows and the reading is taken from the potentiometer dial. Since the lithium cell output is constant the amount that the potentiometer is varied in order to balance the two cells can be related to concentration of the element being determined.

The advantage of the internal standard method is the elimination of differences between behavior of samples and standard solutions due to variable gas and air pressure and viscosity. Since results are an expression of the ratio of lithium concentration to that of unknown, relatively wide fluctuations in atomizing rate, pressures, etc. may be tolerated with no significant change in this ratio. This, of course, would not be true with direct readings without the internal standard.

Certain requirements must be fulfilled by the internal standard before it can correct for the variable factors mentioned. It and the element being determined must have a like response to varied flame temperature, pressures, and presence of other substances; if this were not so, the ratio between the two would obviously be affected. Precaution must be taken to avoid the use of internal standard containing contaminant reference substance. Finally it has been suggested that the analytical and internal standard emissions be approximately equal in intensity.[1]

The principles of flame photometry have been briefly reviewed with particular attention to incorporation of the internal standard into the method. The remainder of this paper will be devoted to a consideration of the equipment, techniques and control of variable conditions that contribute to the maximum accuracy, precision and ease of this method of determination. It will include a description of the way in which we modified our procedure for sodium determinations at the University of Minnesota Hospital chemistry laboratory.

EQUIPMENT

Photometers

The flame photometer used in clinical laboratories must satisfy certain criteria. It must be simple to operate, must give steady and reproducible readings, must be sufficiently sensitive to detect elements in dilute solutions yet remain uninfluenced by other ions, must maintain a steady flame and constant pressures and must be easily cleaned and repaired. The Baird flame photometer used at the University of Minnesota Hospital is one of the machines that fulfills these conditions.

Cells

The photocell is one of the basic components of a photometer. Several types are available: barrier-layer, vacuum phototube and photomultiplier tubes. Barrier-layer cells are independent of external current and give both stability and freedom from fatigue; it is this cell which is used in the Baird machine. For use with an internal standard photocell pairs must be matched (using direct readings) to give maximum and approximately equal galvanometer deflections behind the approximate filters with a given concentration of a standard solution and at a given setting of the sensitivity adjustment.[4] Barrier layer cells are also used in the Barclay and Janke flame photometers.

Filters

Flame photometric analyses are possible only because analytical lines may be separated from other spectral lines by means of filters. There are several kinds: glass, gelatin, liquid and interference. For an extensive discussion and comparison consult Robinson and Ovenston.[5] The amount of interference from adjacent line emission and the minimal detectable concentration of the element for which it is designed vary with individual filters. Inefficient filters are one of the greatest sources of error in photometry; the best obtainable should be employed.

Interference filters transmit a narrower and more specific band of light than glass or gelatin filters do, but they do not separate calcium from sodium emission sufficiently for accurate determination of calcium in solutions containing both. For this reason, of the three interference filters accompanying the Baird machine, only the sodium and potassium filters are recommended for biological material such as serum. The calcium filter should be used for accurate determinations only when sodium is not present in the solutions in detectable amounts.

Barclay and Janke photometers are equipped with single Corning glass or liquid filters. Spectrophotometers using prisms or diffraction gratings and a variable slit width are far superior to filters in wavelength resolution.

Fuel

Emission of more simple spectra and from fewer elements

results from excitation by a gas flame as opposed to excitation by spark in flame or spark gap excitation. Differences in flame temperatures cause variation in emission. High temperature flames produced by burning an oxygen-gas mixture excite more elements but demand more efficient optical systems to separate analytical lines. The Beckman DU spectrophotometer, for example, makes use of an oxygen-acetylene flame and is able to do so because of its superior optical system. The expense of such a machine, however, makes it unavailable to many laboratories. In the photometer class the Barclay uses a propane-air flame while the Janke and Baird instruments use illuminating gas (or natural gas) and air. City gas is readily available in the laboratory so no separate installations are required. For determination of sodium and potassium in biological fluids with a photometer we have found the natural gas-air flame to be satisfactory.

Atomizers

Many styles and materials in atomizers have been used in an effort to meet the following conditions: introduce solution into the flame at a constant rate, require small volumes of sample, permit rapid consecutive determinations, be readily cleaned and noncorrosive. The glass atomizer of the Baird instrument requires only a few milliliters of solution, permits rapid consecutive readings, is readily cleaned and is noncorrosive. However, the rate of introduction of sample into the flame has some dependence upon volume of solution in the funnel. To eliminate this variation we chose to make all standardizations and take all balance readings when the solution was at a specified height (approximately three-fourths full) in the funnel.

TECHNIQUE AND VARIABLE CONDITIONS

Preparation of a Machine

Optimal conditions must be evaluated for each instrument by establishing curves for sodium, potassium and lithium separately, relating output of photocells to ion concentration at different atomizing rates, gas and air pressures and sensitivity adjustments. Conditions are then selected which give maximum precision with reproducibility and stability.

Although much of the discussion to follow applies to flame photometry in general, it is written primarily about the determination of sodium and potassium in biological fluids with the Baird flame photometer.

Sample Preparation and Concentration

Workers agree that determinations for both sodium and potassium are best performed on samples of less than 100 ppm concentration. Unless sodium is kept below 100 ppm linear relationships are not maintained between concentration and emission intensity. One possible explanation for this is self absorption of

sodium emission by cooler atoms.[6] To a lesser extent loss of linearity results when higher concentrations of potassium are used.

Normal human serum sodium level ranges from 137 to 147 milliequivalents per liter (315-340 milligrams percent) and potassium from 4.0 to 5.5 m. eq. per liter (16-22 mg. %). A 1:200 aqueous dilution of serum, bringing sodium concentration to between 10 and 20 ppm provides a good working range. A 1:25 serum dilution for potassium brings the working concentration to between 3 and 14 ppm. Optimal dilutions will be found to differ relative to the sensitivity of the instrument to be used.

Other advantages gained when serum is diluted are that, one, dilution reduces the concentration of interfering elements, making them less detectable;[3] two, dilution increases the amount of solution available for the analysis which in many cases makes possible the analysis of otherwise insufficient quantities of material; and three, obviously a dilution is necessary in order to incorporate the internal standard.

Since potassium will move across the cell membrane upon standing with no visible hemolysis all specimens are centrifuged promptly. Slight hemolysis raises potassium values by 0.1 to 0.2 m. eq. per liter so such specimens are run and the hemolysis is reported. Another specimen is requested if there is gross hemolysis.

Due to a wide range of sodium (1 to 150 m. eq. per liter) and potassium (5 to 50 m. eq. per liter) in urine the water dilutions required to bring the concentrations of unknowns into the range of that of our standard solutions vary. 1:50 to 1:200 dilutions usually suffice for potassium; 1:2 to 1:500 may be necessary for sodium. A rapid estimation for either determination can be performed by taking direct readings on the photometer comparing the response of increased dilutions of urine to that of one of the standard solutions.

Sensitivity

Because in the internal standard method the lithium cell output is balanced by means of a potentiometer against that of the sodium-potassium cell, sensitivity is really adjusted in two ways with the Baird machine: first, by a rheostat in the circuit between the sodium-potassium cell and the galvanometer to regulate output of that cell and, second, by varying the concentration of lithium, thus changing the lithium cell output. A ratio is being measured so a change in either cell output affects the ratio and changes the sensitivity.

Approximately equal outputs from the two cells is preferable. If one of the cells has a much greater response than the other, the influence of changing flame temperature or spray rate will not affect the two to the same degree; a consequence of this, then,

is a galvanometer that is unstable and makes balancing difficult. Furthermore wide variations in the response of the two cells places added demands upon the electrical circuit when balancing them and sensitivity to small changes in concentration is lost.[7]

In preparing a new photometer for the analysis of an element like sodium a sensitivity adjustment should be selected with consideration to both the ion concentration range desired and to the ratio of concentration to potentiometer units. Once the response of the sodium-potassium cell has been selected a lithium concentration can be chosen which gives an approximately equal photocell response. This would also have to be done when new photocells are installed.

Atomization Rate

Air pressure is the most important factor influencing the rate at which sample is introduced into the flame. With dilution and an internal standard factors which affect droplet size and might differ between standards and unknowns (surface tension, density and viscosity) lose their importance.[1] Furthermore, with the use of an internal standard even the velocity of air ceases to be as critical. Fox found that if he reduced the rate of flow in the atomizer from 25 milliliters per minute to three to five milliliters per minute, direct readings suffered a 22% difference but internal standard readings varied less than 2%.[8] As was pointed out in the discussion of atomizers hydrostatic pressure of solution in the funnel of an atomizer such as the Baird uses has some effect on atomization rate. To circumvent this variation we have designated a particular height of liquid in the funnel at which all standardizations and readings are made.

Air Pressure

Change in air pressure has a marked effect on both direct and internal standard readings. This is well demonstrated by White comparing relative readings on the potentiometer dial for the same concentration at different air pressures adjustments. Although both methods were influenced, use of an internal standard showed far greater independence of air pressure.[9] A comparison of the two methods in terms of percent error with deviations in air pressure by Berry, et al, demonstrates again the superiority of the internal standard.[3] Ten to twenty pounds of presure are usually required, depending upon the instrument used.[10,11,12] Cholak and Hubbard point out that the same pressure may not be optimal for more than one element; however, this is something to be determined for each instrument. Ten pounds has been found satisfactory for both potassium and sodium determinations on the Baird at University Hospital.[13]

One can determine the optimum pressure for a machine by

keeping other conditions constant and measuring the emission of a solution at several different air pressure adjustments. A curve obtained by plotting readings against pressure will demonstrate that over a certain range of air pressure there is very little corresponding change in readings. From this plateau one selects the pressure for use because in this range the largest variations in pressure will affect intensity of emission least.

That a change in air pressure does affect the spray rate and consequently the intensity of emission and ultimately the sensitivity was rather dramatically demonstrated at University Hospital when, unknown to the laboratory personnel, the source of air pressure for the Baird was changed from a small air compressor used exclusively by the Baird to a large pressure tank upon which the demand was great and varied. An inadequate pressure regulator between our instrument and the air supply was unable to correct the great variations in pressure and it was impossible to obtain consistent balance readings for the standard solutions. This difficulty was overcome by our system of bracketing.

Bracketing

Bracketing is a procedure in which unknown samples are run in sequence with standards of higher and lower concentrations. Balance readings for the standards are related to their concentration. Then the concentration of the unknown is calculated by the proportional relationship of its balance reading to those of the standards and thus to their concentration. The dilution factor for the sample may be applied before or after the calculation. We prefer to first express the standard concentrations as their actual value times the dilution factor for sample and then calculate the concentration of the unknown directly from these. The following actual example not only demonstrates this procedure but shows that despite air pressure variations replicate readings on the same serum and bracketed between standards showed excellent reproducibility. Had bracketing not been used and, for example, had a predetermined standard curve been used our results would have lacked both precision and accuracy.

Standard 0.8 m. eq./1 × 200 (dilution factor) 160 m. eq./1
Standard 0.7 m. eq./1 × 200 (dilution factor) 140 m. eq./1

Std. 0.7 reads 704
X reads 719
Std. 0.8 reads 810

$$X = 140 + \left(\frac{15}{106} \times 20\right) = 142.8 \text{ m. eq./1}$$

Std. 0.7 reads 727
X reads 732
Std. 0.8 reads 810

$$X = 140 + \left(\frac{5}{83} \times 20\right) = 141.3$$

Std. 0.7 reads 695
X reads 713
Std. 0.8 reads 830

$$X = 140 + \left(\frac{18}{135} \times 20\right) = 142.6$$

Std. 0.7 reads 783
X reads 789
Std. 0.8 reads 865

$$X = 140 + \left(\frac{6}{82} \times 20\right) = 141.6$$

Gas Pressure

Variation in gas pressure does not have the marked effect on readings that variation in air pressure has. White, in terms of relative readings, and Berry, et al, in terms of percent error at different gas pressure levels, showed the relative independence of gas pressure variations that the internal standard method possesses when compared with the direct method.[9,3] However marked pressure change results in error despite the internal standard and a stabilizing device is required.

Again optimum conditions must be individually determined for each machine.

With other conditions constant, obtain readings for the same solution over a range of gas pressures. The curve resulting from plotting these readings against corresponding pressures will present a plateau and a pressure chosen from the range giving this plateau will have the least effect on readings should small variations occur.[11] Our Baird uses a pressure of 2.5 inches of water set with a pressure regulator below the possible fluctuations in the city mains where pressures vary between 3 and 6 inches of water.

Interference

The preceding variables that have been discussed have been conditions readily controlled or for which compensation is made by an internal standard. Interference problems, however, are neither avoided by the most finely adjusted machine or entirely alleviated by the best optical systems. Gilbert, et al, speaking of flame spectrophotometry, but these also arise in photometry, categorized interference types in this manner:[14]

1. Quenching, reabsorption, interference with combustion
2. Energy at other wave lengths
3. Overlapping emission bands
4. Intense adjacent line emission
5. Increase of flame background at all wave lengths

Increased flame background is no problem when using an internal standard. Energy from overlapping or adjacent bands is particularly a problem with filters, less so with a spectrophotometer. If improved filters cannot be obtained and there is interference from this source another analytical line should be sought. Fortunately elements with considerable emission

adjacent to sodium and potassium lines do not occur in great enough concentration in biological samples in which we are interested to cause difficulty.

Interferents may cause negative or positive error, the former by absorption of the emission from test substance, the latter by passage of interfering emission through the filters and registration on the photocell as test substance. Methanol and ethanol, for example, enhance sodium and potassium emission while phosphates, glucose, and urea of sufficient concentration depress both.[2,12] Anions in general depress readings for the two determinations while cations have little effect over a wide range of concentration.[15] As little as 1% sulfuric acid has a depressing effect using either internal standard or direct methods.

It has been suggested that any interfering substance in the unknowns should also be added to the standard solutions. Mosher added to standards for serum sodium and potassium determinations, besides sodium and potassium, calcium, magnesium, ammonium phosphate, glucose, urea, cholesterol and gelatin.[2] This suggestion seems highly impractical, especially since to be entirely correct these components should be first determined for each unknown, then sets of standards made up with corresponding concentrations of the interferants.

The use of a "common matrix" has been suggested, i.e. giving all samples and standard solutions the same major constituent. Since alkalies are known to suppress the spectral lines of other elements, lithium carbonate and like fluxes have been used.[16] The lithium present when the internal standard principle is used may have some effect in this way but its primary contribution to resolution of interference problems is that lithium emission is affected in the same way that sodium or potassium emission is by the interferents.

For most interferences, dilution obviates the problem. Bills, et al, studied the effect of dilution upon interferents and found that for both enhancing and depressing substances the greater the dilution the less the interference despite the fact that the ratio of 1:1000, sodium or potassium to interferent, remained the same.[12]

Probably the greatest interference arises from the effect of sodium and potassium upon each other. Error varies with the instrument, in particular with the optical system. Again dilution appears to be the best solution; even with ratios of 1:1000, the dominant element being the interfering one, Bills, et al, noted marked reduction of error when determinations were made in low concentration ranges.[12] The suggestion has been made to include both sodium and potassium in the standard solutions

and many workers do this. But this provides only an approximate correction. Since our sodium and potassium procedures required different internal standard concentrations the same set of standards made up in such a manner could not be used for both procedures. We depend upon dilution to minimize interference effects.

Because dilution does eliminate much of the error due to interference although the ratio of test substance to interferent remains unchanged a simple means of detecting interference difficulties is to make determinations on a serum in more than one dilution. If results agree for the different dilutions one can safely assume that interference in this range will not be a problem. This would be particularly advisable to do in the case of determinations on urine because of its high concentration of many interferents, in particular, calcium, and because of its varied ratio of sodium to potassium.

Miscellaneous

A few precautions the flame photometer operator should remember include:

1. Allow the machine an adequate time to warm up and excite photocells preliminary to the determinations using any standard solution
2. Remove hand from potentiometer dial before taking readings to avoid grounding the machine
3. Examine machine for faulty contacts if calibration curves drift
4. Avoid poor ventilation; air contaminants like detergent powder and dust can cause erratic results and tobacco smoke places detectable amounts of potassium in the air
5. Soak all new glassware to eliminate the high concentration of soluble alkali usually found on its surface

REVISION OF OUR PROCEDURE

Sodium concentration in serum and urine samples was determined at the University Hospital chemistry laboratory using a total concentration of 125 ppm lithium internal standard in the aqueous dilutions of samples and the same final concentration of lithium in standard solutions of from 0.5 to 0.9 m. eq. per liter of sodium. Unknowns were bracketed (as has been described) between two standards differing in concentration from each other by 0.05 m. eq. per liter sodium.

The sensitivity adjustment was such that when the 0.8 m. eq. per liter sodium standard was atomized and the galvanometer was brought to zero, the potentiometer dial reading was 500 on

a 1000 unit dial. The average difference in balance dial readings per 0.05 m. eq. per liter difference in sodium concentration was 28 units. In other words the sensitivity was at an adjustment at which readings over only one-fifth of the available potentiometer dial included the balance readings for all standard solutions (0.5 m. eq. per liter at 332 to 0.9 m. eq. per liter at 556).

By increasing the sensitivity adjustment we attempted to increase precision by reducing the concentration-to-balance dial reading ratio.[17] An arbitrary balance dial setting of 800 was selected for the 0.8 m. eq. per liter standard and the sensitivity adjustment was altered until the galvanometer was balanced at zero. Using the new sensitivity adjustment the remaining standard solutions were atomized and balance readings were obtained for each. When readings were plotted relating them to concentration a linear relationship was found to exist. 45 dial units corresponding to a difference in concentration of 0.05 m. eq. per liter of sodium.

With the changed sensitivity adjustment, output of the two photocells were no longer approximately equal; consequently reading of the galvanometer was tedious due to its instability. A lithium concentration was sought to equalize the output of the two cells and, after trying both lower and higher concentrations than the 125 ppm lithium previously used, 175 ppm was selected as the concentration giving a suitable compromise between sensitivity and ease of reading.

Precision obtainable at the new sensitivity adjustment and using 175 ppm lithium was calculated. Nine determinations of sodium concentration were made on the same dilution of a serum by bracketing between two standards differing in concentration by 0.05 m. eq. per liter of sodium. Standard deviation in the values obtained was 0.61 m. eq. per liter. Refer to Table 1.

The necessity for bracketing unknown solutions between standards whose concentrations differed by only 0.05 m. eq. per liter of sodium and therefore requiring nine different standard solutions was questioned. Sodium values obtained from ten determinations on the same serum dilution but bracketing between standards whose concentration differed by 0.1 m. eq. per liter of sodium showed a standard deviation of 0.28 m. eq. per liter. See Table 1. Because there was little change in precision when bracketing was performed using standards separated by greater concentration differences a reduction in the number of our standard solutions seemed to be justified. Accordingly we reduced the number of standards from nine (0.5, 0.55, etc. m. eq. per liter) to five (0.5, 0.6, etc.) and adopted the procedure of bracketing between standards differing in concentration by 0.1 m. eq. per liter of sodium.

TABLE 1
Consecutive Determinations on the Same Serum Dilution

Bracketing Between 0.70 and 0.75 m. eq./l Stds.	Bracketing Between 0.7 and 0.8 m. eq./l Stds.
146.8	142.4
146.6	142.4
146.7	142.3
146.2	142.0
146.2	142.0
147.3	142.1
147.5	141.9
145.5	141.5
146.3	142.0
....	141.8
Mean = 146.6	Mean = 142.0
Std. Dev* = .61	Std. Dev = .28

* Std. Dev. $= \sqrt{\frac{\Sigma(x-m)^2}{n-1}}$

A comparison of the sodium determinations before and after the modifications to the method were employed was made. It is the practice at the University Hospital laboratory to keep frozen aliquots of pooled serum and include one aliquot with each set of sodium determinations. Because a range of values has been established for this serum pool, its determination serves as a control for the procedure. Thirty-six values for the control serum chosen at random from work done before the method was modified gave a mean value of 145.1 m. eq. per liter. Thirty-six values obtained for the same pooled serum after modification of the method had a mean of 144.9 m. eq. per liter sodium. This would indicate then that there was no appreciable change in accuracy with the modified procedure.

In precision there was no significant change with the modified method. The standard deviation was calculated using the formula:[18] standard deviation equals $\frac{\bar{R}}{d_2}$ in which $\bar{R}$ represents the mean difference between duplicate determinations on many samples and d_2 is 1.128 when the number of observations per sample is two. Duplicate values were taken at random from actual values obtained in the routine laboratory determinations over a long period of time. They therefore represented work done with as wide a variety of technique as one could expect to find in a laboratory where different students or instructors make the determinations each day. With 50 such values obtained with the unmodified method a standard deviation of 0.51 m. eq. per liter was obtained; seventy-eight values determined with the modified procedure showed a standard deviation of 0.37 m. eq. per liter.

It was felt that performance of the modified procedure would have shown even greater precision had we not had so much difficulty in regulating air pressure after the pressure source was changed. Although precision was not shown to improve markedly, an advantage had been gained by the modification in that the same or a slightly improved degree of precision was maintained while making possible the elimination of half of the standard solutions. Limit of error for the procedure calculated as three standard deviations was approximately 1%. A comparison of precision and accuracy before and after modification should be made following a change in any method.

SUMMARY

The history of the development of flame photometry has been reviewed and the principles of the method discussed. A consideration was then made of the internal standard method, its principles and the advantages of its application.

A comparison was then made of the various components of a flame photometer, stressing the importance of an efficient optical system and noting the equipment in different types of machines.

Preparation of serum and urine samples for analysis was described.

Effect and control of such variables as air pressure, gas pressure, atomization rate and interferences were considered.

Using an actual example of the way in which the method for sodium determination was modified at the University Hospital laboratory, emphasis was placed on some of the considerations necessary before a method may be changed or a new photometer can be prepared for analytical work. Some of the suggestions included may help other clinical laboratories to improve the precision and accuracy of their sodium and potassium procedures, increase the ease with which they do them, or make better use of the capacities of their photometers.

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WE LIKE OUR LABORATORY AIDES*

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Our one-year course for Laboratory Aides here at Amherst H. Wilder Dispensary in St. Paul, Minnesota, has performed an important service for the community since June, 1949. The decision of Dr. Kano Ikeda, pathologist in charge of our laboratory, to begin this course for high school graduates, was based on the findings of a questionnaire sent out in 1947 by the American Society of Clinical Pathologists to all of its members and an equal number of registered medical technologists, chosen at random. Among the questions, there was one pertaining to the establishment of a "Junior grade of medical laboratory worker."

After the replies were analyzed by a qualified statistician, the results of this questionnaire, which had been submitted to a large group of laboratory people, showed conclusively that a large majority wished establishment of this new classification. Consequently the American Society of Clinical Pathologists voted, at their annual meeting in 1947, to establish this certification. A quotation from the American Journal of Medical Technology, January, 1948, page 45, follows: "Junior certification (name not yet given to this group) of medical technologists, the qualifications to be graduation from an approved high school, one year of technical training under the supervision of a recognized pathologist, one year of experience in an acceptable laboratory."

Since we at the Amherst H. Wilder Dispensary laboratory had, for many years, used students of varying educational qualifications to help us do our routine laboratory tests, we decided to follow through with the recommendation of the American Society of Clinical Pathologists. We established here in 1949 a one year course for high school graduates, with better organization than we had previously employed.

Our laboratory aide students are recruited mainly from the local high schools. We prefer girls interested in science and with some proficiency in typewriting. Up to the present we have required no particular scholastic attainment, because we have found that a girl's ultimate efficiency as a laboratory aide does not necessarily depend on her scholastic attainment in high school. Personality traits often are as important as good grades in high school. We aim to take in one new student every three months. They pay no tuition, and neither do we pay them any salary. We have felt that the service they give us is balanced by the technical knowledge they gain. We do give them their lunches for the five work days per week, and the laundry of their

*Read before ASMT, June 1955, New Orleans, La.

uniforms which they must buy. They also buy one large loose leaf notebook and Todd and Sanford's "Clinical Diagnosis by Laboratory Methods" for reference work. They are entitled to a week's vacation, five days of sick leave if they need it, and seven holidays. There is no evening, Saturday, or Sunday work.

On the very first day of their course the students are plainly told their year's training will enable them to do only the routine laboratory tests in a doctor's office or the same tests in a laboratory of a small hospital, under the supervison of a medical technologist. The differences between medical technologists and laboratory aides are clearly pointed out to them. The girls soon learn the glimpses they get of the theoretical backgrounds of complicated laboratory tests are only glimpses, indeed. They realize why there must be differences of responsibility, authority, and salary between the two classifications of laboratory workers. At the same time, the importance of conscientiously doing the routine tests accurately, promptly, and cheerfully is carefully stressed. They learn to organize their work and time, and a sense of responsibility is religiously nurtured. A kind, sympathetic and friendly attitude towards the patients is insisted upon, at all times. Also, each girl learns that she is expected to maintain a friendly and cooperative attitude toward all the other workers during the entire year. Each girl gets four private interviews during the year, at which time her work rating and her personality rating are discussed in detail. These records are kept on file for future consultation, if needed.

The course here at the Amhert H. Wilder Dispensary is planned with a consideration of the patient's tests and needs in first place. That means our laboratory work for the clinics comes first in order of time and importance. Our lessons, study periods, and practice periods must be worked in whenever possible. Our work schedule calls for one class per week for each girl, but she does not always get it on the scheduled day or hour. During this class period we discuss their answers to the study questions I have given them, questions of their own, and any personal matters that need attention. We give the girls definite study questions on each type of work because we feel that less time and energy is wasted in getting the basic facts across. These questions, their lithographed technique notes, demonstrations, and the practice gained in doing the hundreds of tests constitute the main part of the course. We feel that just learning how to do tests is not enough; speed and accuracy can be acquired only by doing hundreds of tests on real patients, many of whom motivate the girls to a great deal of individual scientific study.

The year is divided into four work sections of three months each. The first is mainly taken up in learning urinalysis techniques, including the P.S.P. and concentration and dilution tests.

At the same time the girls get practice in doing Gram's, Ziehl Neelsen's, and Wright's stains, the Benzidine test for occult blood, and practice in recognition of ova and parasites, particularly the Enterobius vermicularis. They do clinic charting, read hemoglobins on the Rouy Photrometer, wash glassware, and observe gastric titrations and basal metabolisms. Sets of study questions on these tests are used at the same time. They get the answers to these questions mainly from Todd and Sanford's "Clinical Diagnosis by Laboratory Methods," though we also have Dr. Opal Hepler's "Manual of Clinical Laboratory Methods" and several other biological science books and pamphlets for reference. I feel that Todd and Sanford's "Clinical Diagnosis by Laboratory Methods" is too advanced for the average high school graduate, but until text books for laboratory aides are written, it shall continue to be our chief text. At the end of her first three months period, each girl is given an oral review plus a written examination on the work she has covered. We feel written examinations are valuable, mainly because of the added incentive toward organized study that they provide.

The second three months period is spent in learning the techniques of finger pricking, blood cell counting, gastric titrations, and basal metabolisms with a McKesson's waterless machine. Toward the end of this second period, the girls are worked into veno-punctures, differential counting, examination of U. and C. smears for G. C., and nasal smears for eosinophiles. They also learn to do Duke's bleeding and clotting times, platelet and reticulocyte counts, and sedimentation rates, both the Westergren and micro. methods. Because of their direct contact with patients suffering from pernicious anemia, sickle cell anemia, erythroblastosis fetalis, polycythemia vera, the leukemias, and infectious mononucleosis, the girls get study questions on these diseases. Then, even though we do not yet do blood groups or Rh factors, we include questions on these tests, because the girls draw the bloods for them on each of our Prenatal patients. This motivates a great deal of interest, both in the theory of the tests and in the progress of the Rh negative mother-to-be. No oral or written examinations on hematology are given until after the third three months period, at which time the lessons and study questions on blood should be completed.

The last three months the students get practice in electrocardiograms and all the tests they have done before, including a review in the urine department. A student, in the last period of her course, further develops her sense of responsibility and organization by taking charge of the laboratory during all of my absences. Study questions, during this last period, are on a few of the more common communicable diseases, using mainly Theresa I. Lynch's "Communicable Disease Nursing" (*2nd* edition).

However, we make use of "Textbook of Healthful Living" by Dr. Harold S. Diehl, Dean of the Medical School, Uni. of Minnesota. "Communicable Disease Control" by Dr. Gaylord Anderson, Director of School of Public Health of the University of Minnesota, and Margaret Arnstein, formerly Associate Professor of Preventive Medicine and Public Health and Director of Course in Public Health Nursing, University of Minnesota; Sharpe and Dohme "Seminars"; Lederle's Bulletin; and "Today's Health." We also use communicable disease bulletins from the Minnesota Department of Health, The Metropolitan Life Insurance Company, John Hancock Mutual Life Insurance Company, Minnesota Tuberculosis and Health Assoc., National Foundation for Infantile Paralysis, and the National Tuberculosis Association. Since laboratory aides in doctors' offices will logically come into contact with the clinical aspect of communicable diseases rather than the technical, this side of the disease is emphasized. The girls do no culture work, but they know what a culture is, and they know the importance of keeping tubes and media sterile. They learn to recognize streptococci, staphylococci, G.C., and acid fast bacilli from smears sent in from the clinics, but they are taught never to report a positive G.C. or acid fast bacilli without first having it confirmed by a medical technologist or pathologist.

Upon completion of this one year course, the girls are given printed Laboratory Aide certificates, signed by the pathologist in charge of our laboratory and the superintendent of the institution. We guarantee no jobs, but we do help as much as possible. Most of the girls have gone into doctors' offices, but a few have gone into hospital laboratories, where they worked under medical technologists.

In order to make a truer evaluation of the course, I have recently sent one type of questionnaire to the doctors who have hired our graduates and a different questionnaire to each of the girls. An analysis of the questionnaires returned by the former students shows some interesting facts.

1. Of the twenty-one girls who enrolled in the course since June, 1949, seventeen finished. Reasons for quitting varied. One girl's board and room money ran out, one girl's father died so she took over the main support of the family, one girl discovered in two weeks she did not like the work, and one girl was asked to leave because she was a personality misfit, and that in spite of her high scholastic standing in high school.

2. Of the seventeen girls who finished the course, ten are still doing laboratory work, three left their jobs for marriage, two have gone into nursing (one immediately after finishing our course), and two have not returned the questionnaire.

3. The sixteen girls who have done laboratory work, have held

a total of twenty-four jobs. Of twenty jobs reported on, thirteen were 40 hours per week, three were 42 hours per week, and three were under 40 hours per week.

4. Laboratory tests done by the girls are mainly routine blood tests, routine urine tests, basal metabolisms, electrocardiograms, smears for G.C., smears for T.B., pin worm examinations, gastrics, and blood sugars. Duties other than laboratory tests include clerical and typing, assistance with patients, telephone duty, appointments, cleaning office, injections, diathermy treatments, ordering, and routine chest X-rays. One girl took care of her doctor's narcotics.

5. Beginning salaries averaged $175 per month, with ten jobs paying less than $175 at the start, and eleven jobs paying more than $175 at the start. At the present time, most of the girls begin at $200 per month.

6. Termination or present salaries average $225 per month, with twelve jobs paying less than $225 per month and nine jobs paying more than $225 per month. One girl received a $100 bonus check each Christmas.

7. The average vacation allowed is two weeks, though three of the jobs give only one week.

8. Under "good points" of their positions, more girls listed "good doctor" than any other comment. Other comments included "good pay," "friendly atmosphere," "satisfaction in work," "good hours," "good working conditions."

9. Under "bad points" of their positions "working late hours" was listed by the most girls. Next came "working every Saturday," "alternating between office and laboratory before completion of work at either place," "poor pay," "no body protection from X-Rays." Three girls said there were no bad points, and seven girls have not left their first positions.

10. Suggestions by the girls on how to improve our Laboratory Aide course include the following points:

a. Eight girls believed we should teach blood sugars.

b. Other suggestions were to teach more medical terminology and more blood morphology, to give greater stress to communicable diseases and urine microscopics, to stress the dangers of contact with diseased persons, and to have more laboratory conferences on patients' problems.

c. Some comments by the girls follow: One girl thinks her job is "just perfect," and another said she "just loved" her job. One girl thanked her "lucky stars" she took our course as she has done for four years just exactly what she had always wanted to do. Another thinks our course is outstanding "as is."

To complete the discussion of the course from the student's point of view, I would like to say that all have been encouraged to attend our local medical technologist meetings, but none have

done so, mainly because they have felt they would be out of place. All have expressed an interest in registration with the Registry of Medical Technologists if registration could be arranged *at the end of their year of training.* No girl will ever feel registration to be advantageous after she has successfully worked in a doctor's office for one year. From her point of view, what could registration do for her? If we, as medical technologists, want these girls registered as certified Laboratory Aides (and we should, if we are to exert any control of the situation), then we should use all our influence to make it possible for them to take their Registry examination immediately at the end of their year of training.

In regard to the doctors' replies to the questionnaires, the following information was received.

1. Of the sixteen girls who went into laboratory work after completion of their year of training, five were reported by their doctors as "satisfactory employees," and eleven were reported as "better than average employees."

2. The doctors credited the girls with such good points as "pleasant personality," "accuracy in work," "conscientiousness," "cooperation," "learned new procedures easily," "good with patients," "absolute honesty," and "kept laboratory in good order."

3. Nine doctors could think of no special way in which they would like to see their girls improve. There was one suggestion for each of the following, "more concentration," "more self confidence," "improve her personality," "improve her efficiency," "more knowledge of blood morphology," and "should continue her education." In answer to the question "Do you feel her faults are due to poor training at the Amherst H. Wilder Dispensary?" two doctors said "Yes," while fourteen said "No."

4. Suggestions for improvement of our course were the following:

   a. Five doctors suggested we do blood sugars.
   b. Four doctors failed to answer this question but they had checked their girls as "satisfactory" or "better than average" employees.
   c. Five doctors were satisfied with the course **"as is."**
   d. One doctor suggested an improvement in veno-puncture technique.
   e. One doctor suggested more knowledge of blood morphology.
   f. As to general comments, one doctor said he was very favorably impressed with his girl and would be very happy to have any of our other graduates at any time. Another doctor liked the way his girl anticipated her work. One generous doctor could see no way in which his

girl could improve, "she has no faults," and he is glad we have the course.

To summarize, I have reviewed our Laboratory Aide course at the Amherst H. Wilder Dispensary in St. Paul, Minnesota. We like our laboratory aides and by "we," I mean all of us who work with them. True, all has not been sunshine on a smooth even pathway, but the dark moments have been only moments, and we feel much good has been accomplished. It has been an open door of opportunity to most of the girls, because most of them could not afford a college education. It has proved to be a boon to the doctors, because, while each needed help, none would have paid medical technologists' salaries for routine laboratory tests. Furthermore, no medical technologist would have been tempted by any of the salaries or any of the jobs. It has seemed to me to be a case of two types of job opportunities with two types of qualified workers needed.

So,—we like our Laboratory Aides! And I think they like us, too!

AMONG THE NEW BOOKS

MEDICAL PARASITOLOGY—LABORATORY MANUAL: by Glenn L. Hoffman, Assistant Professor, Dept. of Bacteriology, School of Medicine, University of N. Dakota, Grand Forks, N. D., 1956. The Burgess Publishing Co., Minneapolis, Minn., 98 pgs. 13 Pictorial Keys. Glossary. References. $2.75.

This is a Manual which could well be in every teaching institution. It supplies an excellent course of study for the embryonic parasitologist. An explanatory note on the use of identification Keys, is followed by a series of check lists and pictorial Keys that simplify the study and identification of the parasites. These latter include the insect carriers of public health importance. The index of clinical diagnosis of diseases caused by protozoa, helminths and fly larvae, the glossary, and reference list all add incentives to further study.

LAB-TROL MANUAL: Dade Reagents, Inc., 1955. Distributed by Scientific Products, Division of American Hospital Supply.

A manual of 24 clinical chemistry methods which may be used with Lab-trol R as a contral in routine work. These have been found to give good results. Some minor modifications have been made and are here passed on to those who wish to use them. Dade Reagents, Inc., 1851 Delaware Parkway, Miami 35, Florida, provides copies of new BLOOD CHEMISTRY PROCEDURES and MODIFICATIONS as they are released.

CLINICAL PATHOLOGY IN GENERAL PRACTICE, from the British Medical Journal. October 1953, to July 1954. (Fully Revised). British Medical Association, Tavistock Square, W.C.I., London, 1955. 39 articles, illustrations and tables. 321 pages. About $3.00.

Although a collection of articles primarily for the practitioner, this book gives excellent background for the interpretation of tests—information which the medical technologist can use for a better understanding of his own work. There are two articles concerned with "Facilities Under the National Health Service" and "The Public Health Laboratory Service" in Britain, with the remainder in sections covering *Practical Techniques, Renal and Alimentary Function, Infections, Hematology, Metabolic and Endocrine Disorders,* and *Special Problems.*

CARDIAC CATHETERIZATION*

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Since all of you here today are, in some way, concerned with laboratory methods, cardiac catheterization should be of general interest, not only from the dramatic standpoint of "passing a tube into the heart," but also from the point of view of the technologist, who plays an important role in this laboratory procedure.

The purpose of this paper is not to describe in detail the analytical procedures connected with cardiac catheterization, for that would be of interest only to those actively engaged with them, but instead is to point out general methods and what can be learned from these methods.

You might ask, "What is cardiac catheterization and what are the indications for its use"? It is a laboratory procedure in which a small flexible tube is passed into the heart by way of a peripheral vein. It has achieved its greatest recognition from the public in its use in the diagnosis of congenital heart disease, particularly in the case of "blue babies."

In spite of the fact that cardiac catheterization has been recognized as the valuable adjunct that it is in only recent years, it is not new. It was first performed in 1929 by Forssmann. Since people still face the procedure with fear and trepidation, even after it has been fairly well standardized and proven reasonably safe, imagine the courage of Forssmann who volunteered to be the first subject for his own experiment. It is even more commendable when we learn that his assistant refused to pass the catheter in him and Forssmann was forced to do it on himself while watching the course of the catheter on the fluoroscope by means of a mirror.

As with most investigative procedures in our mechanized age, cardiac catheterization requires considerable technical equipment. The quality and the quantity of equipment required, in my experience, seems to vary in direct proportion with the funds available. Scientists, for obvious reasons, seem happiest when surrounded by a maze of dials, knobs, switches and vacuum tubes, and I must confess this as one of my weaknesses.

* Read before ASMT Convention, June, 1955, New Orleans, La.

The central piece of equipment is, of course, the catheter. These are woven nylon, plastic coated and opaque to X-rays—the same composition as ureteral catheters. They are 100 cm. in length (125 cm. for large adults) and their caliber is designated numerically just as with ureteral catheters—6, 7, 8 & 9 French. The proximal end is fitted with a female Luer-lok adapter. The distal end terminates in a blunt tip.

Since catheterization is done under fluoroscopic control, a horizontal fluoroscope or an operating table outfitted with a fluoroscope is the next essential.

To measure pressure through the catheter, one may use a simple water or mercury manometer; however, the results thus obtained are mean pressures and give no information as to systolic or diastolic pressures or of pulse contours. To obtain this information, graphic recording equipment must be resorted to. This is where the budget really can be stretched, if desired. Recording manometers are of several types; (1) optical, in which fluid pressures proportionately displace a membrane on which is mounted a mirror, which in turn displaces a light beam on photo-sensitive paper, and (2) electrical, in which the fluid pressures are converted into electrical intelligence by changing the resistance, capacitance or inductance of the manometer, depending on the type of manometer in use. This electrical information is fed to a galvanometer which deflects a light beam or writing pen in direct proportion to the strength of the signal.

A direct writing electrocardiograph wired to the patient is very important so that any cardiac irregularities caused by the catheter may be detected immediately. A slightly more elaborate set-up is an oscilloscope upon which the ECG may be observed at all times, just like on a television picture tube, without the necessity of running off yards and yards of recording paper.

The equipment thus far mentioned, together with needles, syringes, a few surgical instruments, sterile drapes, etc., constitute the basic equipment. Other instruments may be required, depending upon the type of information being sought by cardiac catheterization.

Before going into the technic, let's review briefly the course of the circulation. (Figure 1) Venous blood returns to the heart by way of the superior vena cava from the head and upper extremity and by way of the inferior vena cava from the lower extremities and abdomen. These two vessels empty into the right atrium. Blood from the right atrium then goes through the tricuspid valve into the right ventricle, then through the pulmonary valve into the man pulmonary artery. From here, the blood is distributed to the lungs where it picks up its oxygen supply. The pulmonary veins then collect the blood, returning it to the heart, this time to the left side. From the left atrium, it

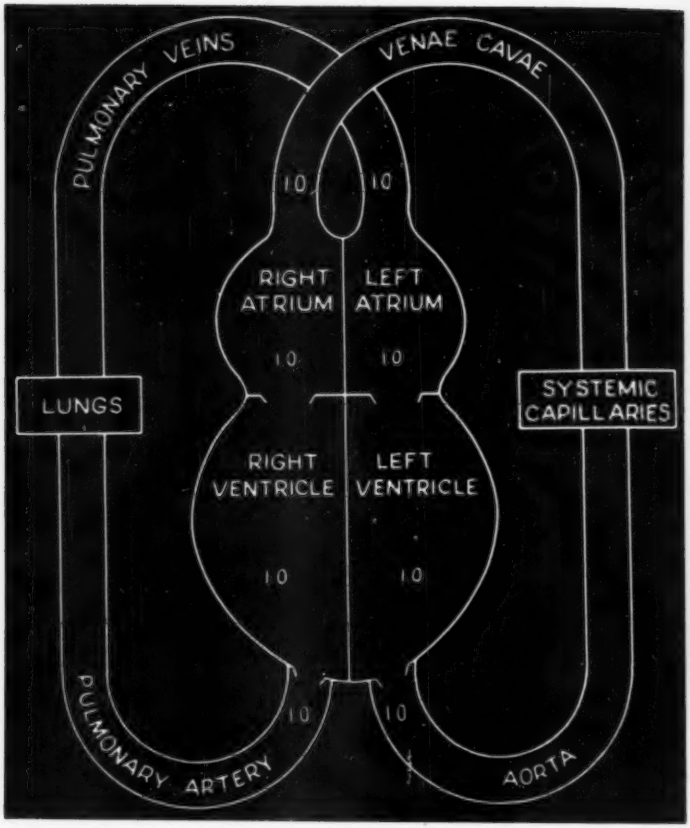

Figure 1

goes through the mitral valve, into the left ventricle, then through the aortic valve into the aorta whence it is distributed to the arterial side of the circulation.

The technic of introducing the cardiac catheter is comparatively simple. The patient lies in a supine position and should be made as comfortable as possible so that near basal conditions may be established. General anesthesia is not required. A cutdown is done over the medial antecubital vein after infiltrating the skin with a local anesthetic. A small nick is made in the vein

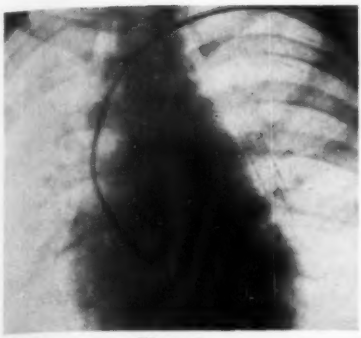

Figure 2

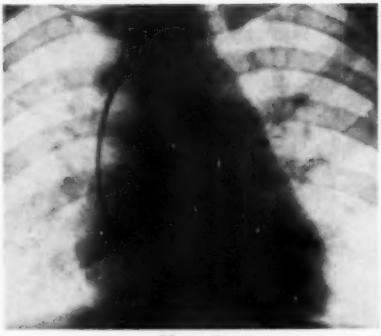

Figure 3

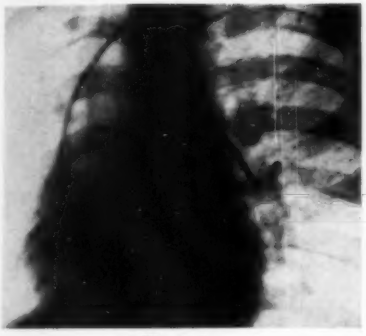

Figure 4

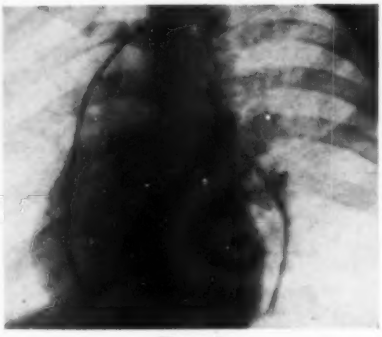

Figure 5

and the catheter is introduced through this hole. It is then threaded up the vein to the heart, following the course of the circulation, while an infusion is kept dripping through the catheter to keep it from becoming stopped up by clotted blood. With an observer watching the electrocardiograph and the operator watching the fluoroscope, the catheter is manipulated into the various desired locations. Since the distal end of the catheter has a curve in it, rotating the proximal end gives some control over which way the catheter advances.

These next photographs show in sequence the position of the catheter at different stages during the procedure. In Figure 2, you can see the catheter as it courses from the arm to the superior vena cava, with the tip lying in the right atrium. In Figure 3 the tip has been advanced into the right ventricle and lies in a position just beneath the pulmonary valve. Figure 4 shows the tip of the catheter in the left pulmonary artery. Figure 5

shows the patient in a slightly different position. This was done to show better the position of the catheter. You will note that there seems to be a lot of catheter lying out in the lung field. Actually it has been advanced as far as it will go and is jammed or wedged into the tapering pulmonary arteriole. Blood drawn from this position is pulmonary capillary blood and is fully oxygenated.

Now that we have the catheter in place, how do we obtain any information from it? In general, there are four different ways that the catheter yields information.

The first of these is by the physical presence of the catheter itself. For example, suppose the tip of the catheter entered the right atrium, then immediately went out into the right lung field. This would suggest the presence of an anomalous pulmonary vein draining into the right atrium instead of into the left as it should. Or suppose the catheter entered the right atrium and then crossed directly over to the left side, emerging in the left lung field. This would suggest a hole in the septum which separates the right and left atria—an interatrial septal defect. Or suppose the catheter went from the right atrium to the right ventricle, and then into the aorta instead of the pulmonary artery. This, of course, would be indicative of a defect in the interventricular septum.

You will note that the position of the catheter *suggests* a diagnosis. Additional information is certainly to be desired. For this, we resort to the second method of obtaining information—the analysis of the gas content of blood samples taken from the various locations in the heart and vessels.

Blood is drawn anaerobically from the catheter in syringes in which the dead space has been filled with heparin solution. The syringes are capped and put into an ice bath. Analysis of the blood for its oxygen content is done as soon as possible and may be done in any of several ways—The Van Slyke manometric, the Scholander-Roughton volumetric, the colorimetric or the polarigraphic method may be used. The results are expressed in cc's of oxygen per 100 cc's of blood, or volumes per cent.

A normal individual might be expected to have 12 to 14 vol. percent of oxygen in mixed venous blood before it gets to the lungs to replenish its supply. In the same individual, blood from the lungs, arterial blood, would have 18 to 20 vol. percent of oxygen.

If the oxygen content of blood samples taken from the superior vena cava, inferior vena cava and right atrium were found to be approximately the same, but the oxygen content of the blood from the right ventricle was found to be 2-3 vol. percent higher, this would clearly indicate that blood from the left side of the heart (highly oxygenated blood) was getting to the right

side at the ventricular level. From this evidence, one could tentatively diagnose an interventricular septal defect.

Similarly, if the oxygen content of the right atrium and right ventricle were the same but that of the blood from the pulmonary artery was higher, a shunt of arterial blood from the left side into the venous blood on the right could be diagnosed at this level, the most common of which is patent ductus arteriosus.

Not only is it possible to locate the site of a shunt by this method, but one may also calculate with a reasonable degree of accuracy the actual volume of blood that is shunted from one side to the other. Suffice it to say that it can be done, so that we don't get involved in mathematics, even though the formula is comparatively simple.

The third method of obtaining information with a cardiac catheter is the measurement of pressures. The most popular type of manometer in my experience is the strain gauge. With this manometer, a very small volume displacement—approximately .01 cubic mm.—alters its electrical resistance and produces a corresponding deflection of the galvanometer used for recording.

If the tip of the catheter is in the main pulmonary artery and the systolic pressure at that point is found to be lower than the expected 25 mm. Hg., one might surmise that the right ventricle is not contracting with sufficient force or that there is a restriction to flow between the right ventricle and pulmonary artery. If the catheter is then withdrawn so that the tip lies in the right ventricle and the pressure here is found to be higher than the expected normal of 25 mm. Hg., the diagnosis of resistance to outflow from the right ventricle—or stenosis of the pulmonary valve—is confirmed.

The fourth method consists of injecting various substances through the catheter. One such substance is a radio-opaque liquid such as Diodrast. A quantity, up to 50 c. c., is injected very rapidly. Immediately, serial radiographs are taken in very rapid succession. From these X-ray pictures, it is possible to see localized abnormalities as well as to determine any abnormal course of the circulation. This procedure, however, has definite limitation. Dyes, such as Evans Blue, are also injected through the catheter. By measuring electrically the light transmitted through the pinna of the ear, one can determine exactly the time that the dye-containing blood arrives at the ear and the quantity of dye in the blood traversing the ear at any particular instant. Using this technic, cardiac output may be determined, again mathematically. It is also useful in determining circulation time from various points in the heart, a valuable procedure in detecting shunts from the right side of the heart to the left. If the circulation time from the right ventricle to the ear was found

to be 9 seconds but the time from the right atrium to the ear, a greater circulatory distance normally, was only 5 or 6 seconds, this would indicate that the dye must be getting to the left side at the atrial level—an interatrial septal defect with the shunt from the right to the left.

There are many other things that may be learned from cardiac catheterization. Probably the most reliable method of determining cardiac output is with the Fick principle in which mixed venous blood is obtained from the catheter at the same time that a blood sample is being drawn from a peripheral artery and oxygen consumption is being determined. The calculation is made using the oxygen consumption per minute and the difference in oxygen content between the arterial and venous blood samples. Much has been learned about pulmonary physiology by the methods that have been mentioned as well as by the analysis of gas tensions in blood samples obtained.

With the remarkable advances that have been made in surgery on the heart, cardiac catheterization has been a boon in accurately establishing diagnosis preoperatively and in assessing post-operatively the results of surgery.

It has opened up new horizons in cardio-pulmonary physiology —fields which are yet to be explored, exploration of which I hope many of you, as technologists, are, or will be, participating as vital members of the team.

PITFALLS TO BE AVOIDED IN CYTOLOGIC DIAGNOSIS*

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Every laboratory procedure used for medical diagnosis has certain conditions which must be met to assure the accuracy of the method. The cytologic method of Dr. George N. Papanicolaou for the diagnosis of cancer is the one laboratory procedure upon which we may rely for diagnosis in some cases, and the only method for picking up unsuspected early malignancy in many others. It therefore behooves each of us engaged in this work to make his results as accurate as is humanly possible. It goes without saying that special training in the techniques for preparing and screening the smears is essential. We would like to review today some of the booby traps which the cytologist must avoid in order to maintain the high level of efficiency that can be expected from cytologic diagnosis.

When smears are made by other individuals and sent to the laboratory, they have already been put into the fixative. There is little the technician can do in these cases to correct errors that may already have been made. He can, however, refuse to accept smears that are obviously too thick or too thin. But he can only pray that apparently good smears were not allowed to dry before reaching the fixative.

In smear preparation there are a number of unpleasant things that eventually seem to happen to all of us. Somewhere along the road of accumulating experience, each of us has had the horror of using inadequate amounts of albumin or of handling a slide too vigorously. Then comes the woe of discovering that all the material has slipped off the slide. There are the over rushed days when the pressure of work and disturbances result in overstaining or understaining. (Which of these occurs will depend on exactly at which point the telephone rings.) Occasionally we forget to filter the hematoxylin, or discover too late that the hematoxylin or the EA have suddenly become most uncooperative. These slips in preparation of the smears have caused many headaches to the screeners who must always be on the alert for their possible occurrence.

We will now dwell briefly on some of the pathological conditions that can throw the screener into a state of diagnostic turmoil. Regardless of the source of the material being investigated, the possible presence in the patient of chronic nonspecific infection must always be borne in mind. Chronic infection is capable of producing amazing nuclear enlargement and cytoplasmic vacuolization that may lead to over evaluation of the smear.

* Read before ASMT Convention, June, 1955, New Orleans, La.

Bronchiectasis is one infectious, hypertrophic condition which cytologically produces annoying clusters of cells with small but often hyperchromatic nuclei. These clusters must be very critically inspected in some instances to differentiate them from malignant clusters.

Now we come to the specific infection that is the bug-bear of the vaginal smear—Trichomonas vaginitis. The Trichomonad is a very remarkable, confusing, unreliable, and I might add, unpleasant little fellow. If he were always present in great numbers and could be counted upon to stain well, we might remember to be on guard against the cytologic changes he is capable of producing. The excellent monograph of Bechtold and Reicher entitled "The Relationship of Trichomonas Infestations to False Diagnoses of Squamous Carcinoma of the Cervix" has done much to shed light on this complicated and often aggravating situation. We know that the changes caused by the Trichomonads are reversible after the organisms have been destroyed. There is still, however, the critical interval between therapy and normality that may befuddle the screener who has inadequate history of the case.

Another specific vaginal infection which may cause confusion is Moniliasis. A long standing Monilia infection can produce cytologic atypia similar to those caused by the Trichomonad. A good general rule is to defer final opinion on smears showing these infections until at least a month after the patient is negative by fresh smear.

A pathological condition we fortunately do not encounter too often is calculi. The presence of calculi in any tissue produces chronic irritation which results in what we cytologically term "active" nuclei. On finding many crystals in a urine sediment along with RBC's, we may become alert to the possible presence of stones. This, however, is not a dependable criterion. Pulmonary calculi never warn the cytologist of their presence. You can readily visualize the difficulties which might result from calculi and superimposed infection. (Such a situation was once my own personal Waterloo.)

Any pathological condition which can cause hypertrophy or hyperplasia may send the overzealous cytologist down false trails. Now let us discuss the normal cells that may act as sources of difficulty.

The presence of a great number of red blood corpuscles may cause understaining. They may also cause great dilution of the other cellular components of the smear. In our laboratory we make it a practice to overstain with hematoxylin every smear showing gross blood. Several methods have been suggested to layer body fluids in order to pipette off the red cells. We have had little success with them. Dilution of cellular material is also

caused by pus cells in great numbers. Often it is wiser in these cases to inspect several extra slides made from such material.

Histiocytes, particularly in clusters, can be extremely unpleasant. Their bizarre shapes, cytoplasmic inclusions, vacuoles, and active nuclei must be very carefully evaluated. (I have seen some clusters of histiocytes that did their worst to convince me that they were malignant endometrial cells.)

The method of collection of a specimen should always be known to the screener. A urine sediment collected by ureteral catheter contains cell clusters from the renal pelvis. These clusters often have large, deep-staining nuclei, and indistinct cell boundaries which may be troublesome to the uninformed screener. Bronchial washings may cause untold difficulties unless the cytologist is well aware of the bizarre pictures the ciliated cells of the bronchial epithelium can produce. One does well to hold fast to Dr. Papanicolaou's rule that ciliated cells are never malignant. Clusters of vacuolated mesothelial cells in pleural and peritoneal fluids must be carefully evaluated and then disregarded. The presence of great numbers of lymphocytes in body fluids should never be overlooked, and should always be reported because they are often diagnostically significant. Perhaps the most unpleasant cytologic situations we have saved to mention last. These are the abnormalities produced by radiation and abortion. We cannot overemphasize the need to the cytologist for histories in cases such as these.

We purposely have omitted discussion of faulty collection of material. Wherever slips in technique are suspected, the experienced technician will reject the specimen and defer diagnosis until a properly obtained specimen has been submitted. Many laboratories, for example, will not give any clasification on a sputum in which numerous histiocytes are not found. The cytologic diagnosis of cancer is an excellent and valuable procedure when its optimum conditions are met. The specimens must be properly collected, the histories adequately recorded, the slides carefully made and correctly stained.

In conclusion, we would say that the aforementioned pitfalls are not pitfalls at all to the wary, experienced cytologist. In time weighing, rejecting, and accepting criteria for malignancy become relatively automatic, like gear shifting. Every student of cytology has at one time or another been exposed to the old cytologic adage which never, never fails to develop a cautious screener. "Don't stick your neck out!"

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AMONG THE NEW BOOKS

THE HEMORRHAGIC DISORDERS—A Clinical and Therapeutic Approach by Mario Stefanini, M. D., Associate Profesor of Medicine, Tufts University Scohol of Medicine; Director of Research Laboratories and Hematologist, Saint Elizabeth's Hospital, Boston; Established Investigator, American Heart Association, and William Dameshek, M.D., Professor of Medicine, Tufts University School of Medicine; Senior Physician and Director, Blood Research Laboratory, New England Center Hospital, Boston, 1955. Grune & Stratton, New York & London. 368 pages. 147 illustrations, including charts and color photographs. 36 tables. $10.00.

The 49-page appendix *Technics in the Study of Hemorrhagic Disorders* will be of most interest to the medical technologist. Those used in the preliminary investigation of "bleeding problems" are described in detail. The bibliography of 753 references includes those to more complicated tests. Two tables are included in this chapter, namely: "Laboratory Investigation of the Bleeding Patient—Screening and Specific Tests," and "Screening Tests"—giving the test and significance of abnormal results.

"The Hereditary Aspects of the Hemorrhagic Disorders" is another chapter of considerable interest to the medical technologist.

TECHNIQUES IN BLOOD GROUPING: by Ivor Dunsford, Senior Scientific Officer, Regional Blood Transfusion Center, Sheffield Lecturer, Scheffield College of Technology, and C. Christopher Bowley, M.B., B.S., M.R.C.O.G., Director, Regional Blood Transfusion Center, Sheffield, Hon. Lecturer to the Department of Pathology, University of Sheffield, with a preface by R. R. Race, F.R.S., Director, Medical Research Council, Blood Group Research Unit, London, 1956. Oliver and Boyd, Edinburgh, London and Charles C. Thomas, Publisher, Springfield, Illinois. 220 pages. 26 illustrations. $4.50.

This is a very practical manual for the medical technologist specialist in the blood bank or in the general laboratory. As stated in the introduction, even the spiral wire binding which allows the book to lie flat without propping, increases its usability. We agree with Dr. Race who says in the Preface, "The expert, however set in his ways, cannot fail to find much of interest—but the beginner—and the director beginning to organize a new blood grouping laboratory will find it invaluable."

Following 14 chapters on Principles, Factors, Methods, Apparatus, Organization, Diagnosis, and Teaching, there is the section with 80 detailed techniques. The Glossary, which makes up Section III has more than 500 terms. We can recommend this volume for its usefulness to any laboratory library.

CARBON DIOXIDE AND OXYGEN DETERMINATIONS USING THE VAN SLYKE-NEILL MANOMETRIC APPARATUS*

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One of the most important functions of blood is to transport oxygen to the tissues and carbon dioxide from the tissues to the lungs. It frequently becomes necessary to determine accurately the content of these gases in the blood and it is the purpose of this paper to review the methods, techniques, possible sources of error in the tests for carbon dioxide and oxygen, and to discuss the maintenance of the Van Slyke-Neill apparatus.

A volumetric apparatus and method for the determination of carbon dioxide and carbonates in solution was first introduced by Donald D. Van Slyke in the Journal of Biological Chemistry in 1917. The use of a manometric apparatus, which is probably more widely used today, was reported by Van Slyke and Neill in this same Journal in 1924. The chief difference in these two machines is that in the volumetric apparatus the pressure of the gas is brought to atmospheric and the gas volume read on a scale. In the manometric apparatus the gas volume is brought to an arbitrarily chosen level (0.5 or 2 ml.) and the amount of gas is determined from the pressure exerted on the manometer.[1] The gas to be determined is then either ejected from the machine or absorbed by a suitable chemical and a second reading is taken on the manometer. Multiplying the difference in these two readings by a factor gives the volumes percent of the gas to be determined when the gas is ejected. For more accurate results the gas may be eliminated after the first total pressure reading by using a solution that will absorb it. This absorbing solution will cause a lowering of the mercury in the chamber and manometer and the amount of this lowering, determined by a blank analysis, serves as the correction factor to be subtracted from the difference in the two pressures. The calculation when the gas is absorbed is:

$$\text{Pressure Vol.\%} = \underset{\text{total gas pressure}}{P_1} \quad \underset{-}{\text{minus}} \quad \underset{\text{pressure after absorbing}}{P_2} \quad - \text{C X Factor}$$

The factor was developed by Van Slyke and Stadie in 1921 when they determined the solubility of carbon dioxide, the temperature effect on the specific gravity of mercury, and the vapor tension of water using varying amounts of solution and testing

* Reviewed in the Veterans Administration and published with the approval of the Manager, VAH, West Haven, Conn. The statements and conclusions published by the author are a result of her own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

** Read before ASMT, June, 1955, New Orleans, La. Third Award, 1955, Scientific Products Foundation, Biochemistry Division.

substance. The factor has now been adapted to the manometric apparatus and is a function of the temperature.

Bloods drawn for the determination of oxygen and carbon dioxide should, of course, be drawn and handled anaerobically. Specimens of whole blood may be drawn into a syringe in which the plunger and inside of the barrel have been coated with a solution of heparin containing sodium fluoride as a preservative. This coating prevents clotting of the blood and occludes the dead space between the plunger and barrel of the syringe. When the syringe is well coated the excess is expelled through the tip so that there is no air in the syringe and a drop of mercury is added to the syringe for mixing. After the blood is drawn the syringe is stoppered with a metal closure or with a needle the tip of which has been imbedded into a rubber stopper. For whole blood oxygen or carbon dioxide determinations the sample may be left in the syringe and mixed by rotating. If it is not possible to complete the test immediately it should be stored in ice water in the refrigerator; then warmed and well mixed before use. The blood should not be used when it is colder than room temperature because one degree centigrade difference in temperature between the sample and the water jacket will cause an error of 0.1% in the carbon dioxide content because of the effect of temperature on the solubility coefficient of carbon dioxide.[2] Samples of blood drawn for plasma or serum tests should be drawn under oil and the carbon dioxide will not escape to any extent until the tube is centrifuged. Oil retards, but does not completely prevent the escape of carbon dioxide from the surface of the blood.[3] Blood should be centrifuged in a tube completely filled with blood so that there are no air bubbles, or under a layer of oil in a constricted tube, or under a layer of paraffin.[4]

Van Slyke and Neill in their article describing the manometric apparatus state that of the details essential to the precision attainable with this apparatus none is more important than accuracy in sampling and in the introduction of samples into the analysis chamber.[5] Ostwald stopcock pipettes calibrated to deliver between marks should be used and should be provided with a rubber tip so that the pipette will fit tightly against the neck of the cup and no air will leak around it. With samples of whole blood care must be taken in transferring the blood from the syringe to the pipette. A piece of rubber tubing may be fitted to the tip of the syringe and some blood is wasted through the tubing. It is then attached to the pipette and blood pushed through the tip of the pipette to the upper mark while holding the pipette in a vertical position. When the pipette is removed from the tubing the rubber tip is put in place so that the blood in the bottom of the pipette never comes in contact with room air. Or the stopper on the syringe may be replaced by a short

20 to 25 gauge needle and the blood transferred to the pipette through the needle and rubber tip.

The solutions used in all these determinations are the same although the amounts of the components may vary depending on the author's experience. Carbon dioxide of the blood is chiefly in the form of bicarbonates and the oxygen exists primarily as a hemoglobin compound in the red cells. In order that the pressure of these gases may be measured it is first necessary to release them from these compounds. The solutions used are saponin and potassium ferricyanide to lake whole blood and release oxygen, lactic acid to decompose bicarbonate and release carbon dioxide. During the determinations sodium hydrosulfite is used to absorb oxygen and sodium hydroxide to absorb carbon dioxide. Because absorbing solutions are used after the blood or serum has been added to the machine they must be made air free and stored anaerobically before starting the tests. Solutions are made air free by admitting an amount to the machine, sealing, lowering, and extracting two or three times. The solution is shaken, the air expelled, and the solution lowered and shaken again until no air bubbles are formed. By placing the mercury bulb on the upper support the solution will rise from the chamber into the cup and directly into the storing device.

These solutions may be stored in several ways. A calcium chloride tube may be fitted with a piece of rubber tubing and a clamp which connects with a piece of glass tubing and a rubber tip. The solution is protected by a layer of oil. The gas free solution may be stored in a special 30 cc. syringe with a glass tube and rubber tip. The tip of the syringe is set in mercury as a seal. Larger amounts of gas free solution may be stored under mercury in a modified Hempel pipette or in two leveling bulbs one of which has a glass capillary stopcock fused to it. One of the bulbs is half filled with solution and the other filled with mercury acts as a levelling bulb. They are connected by a short piece of heavy walled tubing. Solutions may be rendered gas free by evacuating, shaking, and ejecting the gas. If the solution is protected by oil it must be used the same day, but if completely protected by mercury it may be used a week but not longer.[6]

When these gas free solutions are admitted to the machine an extra ml. of solution is measured into the cup and then wasted so that the top ml. that has been exposed to room air is never used in the test. Also the tip of the storing device is set at the bottom of the cup and under the level of the fluid when delivering solution so that the cup fills from the bottom and the bottom ml. of fluid has not been exposed to room air. After the required amount of solution is added from the cup to the extraction chamber a mercury seal is always placed in the stem of the cup. The seal serves a dual purpose in that it keeps the apparatus air tight

and also forces the drop of fluid in the stem of the cup into the machine. Caprylic alcohol is used in all these tests to prevent foaming.

Probably the most common use of this apparatus is in the determination of serum or plasma carbon dioxide, and the same method and principle is valid for whole blood carbon dioxide.[7] The original method for serum carbon dioxide was for the capacity determination in which the serum is exposed to carbon dioxide and the amount then determined. However, the most complete information concerning the acid base balance of the blood is obtained by determining the carbon dioxide content.[8] To measure the content one must be careful to avoid the addition of carbon dioxide from the air. Caprylic alcohol to prevent foaming, lactic acid to decompose bicarbonates and release carbon dioxide plus the blood plasma or serum are admitted to the chamber, sealed, lowered, and shaken for three minutes. The volume of gas is reduced to 2 ml. and the pressure exerted read on the manometer. The gas is then ejected and the solution returned to the chamber and P_2, the zero pressure, is read with the solution again at the 2 ml. mark. For greatest accuracy the carbon dioxide is absorbed by air free sodium hydroxide instead of being ejected. When the solution is ejected the carbon dioxide content is calculated by subtracting P_2 from P_1 and multiplying by the temperature factor. When the carbon dioxide is absorbed the calculation is $P_1 - P_2 - C \times$ factor. Readings are always made by leaving the leveling bulb on the lower support and the solution must be carefully brought to the 2 ml. mark by regulating the bottom stopcock without oscillation particularly in carbon dioxide determinations. If the mark is not reached the solution should not be readjusted but lowered and shaken again and then read. The temperature factor takes into account some reabsorption of the carbon dioxide but, if the fluid is moved up and down in the chamber, more reabsorption of carbon dioxide will occur than is allowed for in the factor.[9]

Oxygen determinations must, of course, be done on whole blood because it is bound in oxyhemoglobin according to the reaction:[10]

$$\underset{\text{Hemoglobin}}{Hb.} + \underset{\text{Oxygen}}{O_2} \rightleftharpoons \underset{\text{Oxyhemoglobin}}{HbO_2}$$

Arterial blood is usually used for these determinations because the oxygen content of venous blood varies greatly and does not reveal as true a picture of the oxygen content of the blood as does arterial blood which has been aerated by the lungs and has not given up its oxygen to the tissues. The blood is laked and the oxygen released by a solution of saponin and potassium ferricyanide. The carbon dioxide is absorbed by sodium hydroxide and the oxygen is absorbed by a solution of hyposulfite.[11] The saponin potassium ferricyanide solution will react with

mercury; therefore when it is shaken to render it air-free the mercury solution interface should be brought well down below the 50 ml. mark where the diameter of the chamber is small and there is a minimum of contact between mercury and solution.[12] Oxygen absorber may be made in a pure solution of hydrosulfite, but most directions include anthraquinone beta-sulfonate. This is not absolutely necessary but acts as a catalyst so that the absorption may be completed in one minute whereas three to four minutes would be required by hyposulfite alone.[13] The powdered contents of the absorbed may be mixed and stored and weighed out each time. The absorber is quickly mixed in 1 N potassium hydroxide with a stirring rod and filtered through a funnel containing cotton into the cup of the apparatus and allowed to enter the chamber directly. This process should be carried out as quickly as possible to avoid oxidation.[14] The normal values for oxygen content in arterial blood are 18-20 vol.% and for venous blood 12-14 vol.%.[15]

Whole blood oxygen and carbon dioxide may be done on the same sample by combining these methods.[16] The blood is shaken in the reaction chamber with a solution of saponin, potassium ferricyanide and lactic acid which will release both oxygen and carbon dioxide (P_1). The carbon dioxide is absorbed by sodium hydroxide (P_2) and the oxygen by hydrosulfite (P_3).

$$P\,CO_2 = P_1 - P_2 - C \times \text{Factor}$$
$$P\,\ O_2 = P_2 - P_3 - C \times \text{Factor}$$

The saponin, potassium ferricyanide solution and lactic acid solution are made separately and mixed in equal volumes each day because the acid mixture gradually decomposes and some of the products settle as a blue precipitate.[17]

The oxygen capacity of blood may be determined by exposing the sample to room air until the hemoglobin has taken up all the oxygen possible. Then an oxygen content is done on this aerated specimen to determine the capacity. The content divided by the capacity multiplied by 100 will give the percent saturation of that blood. It has been established that 1 gm. of hemoglobin is capable of combining with 1.36 ml. of oxygen so that the capacity in volumes percent may be divided by 1.36 to give the hemoglobin content of the blood.[18] Because this is the most accurate method of determining hemoglobin it is used for the calibration of hemoglobinometers and used to establish a calibration curve for any hemoglobin method.

The apparatus is cleaned between tests by ejecting the solution, lowering the mercury to the bottom of the chamber, and adding 10-15 cc. water with about 1 cc. 1N lactic acid. The chamber is shaken for a few seconds and the gas and solution are ejected. The lactic acid remaining on the walls of the chamber does not contain enough gas to interfere in the next test.[19]

In cleaning the apparatus between oxygen determinations where ferricyanide solution has been used it is well to add a few drops of alkaline hydrosulfite to the first water admitted to the machine.[20] We have found that if the unused portion of the oxygen hydrosulfite absorber is left in the cup it may be run into the machine with about 6 ml. of water as the first step of the cleaning process and then followed by lactic acid and water. When all the tests for the day are completed 1N sodium hydroxide may be run into the chamber to dissolve any remaining particles and then washed with lactic acid and water.

When it becomes necessary to clean the entire apparatus all the mercury is withdrawn into the bulb. Solutions may be run into either side of the apparatus by manipulating the stopcocks. To force solution into the manometer close the stopcock to the chamber and the center waste stopcock and open the manometer and lowest stopcocks and the solution will enter the manometer from the bulb. To clean the other side close the stopcock on top of the manometer and the center stopcock and open the stopcock to the chamber and the solution will enter from the bulb. The manometer may be disconnected from the chamber and bulb, of course, and cleaned as any piece of glassware. If the manometer is extremely dirty it may be cleaned with an alcoholic potassium hydroxide solution and then acetone and air or chromic-sulfuric acid solution and air. It is also possible to disconnect the leveling bulb and attach a tube from a suction flask to the bottom stopcock and draw solution through the manometer from the top.[21]

One of the most important sources of error is leakage of air into the machine. Obviously, if one wishes to measure the amount of oxygen or carbon dioxide in a sample leakage of room air which consists of 20.94% oxygen, 0.03% carbon dioxide and 79.03% nitrogen can cause a sizeable error.[22] Proper care of the stopcocks will eliminate this error. Incomplete lubrication should never be tolerated for a single analysis in either of the cocks.[23] The cock at the top of the chamber may be easily greased by closing the bottom stopcock and removing the one to be greased. Before greasing the top stopcock water should be run into the chamber so that after the greasing the water may be forced out of the chamber and will remove any excess stopcock grease. We clean the stopcock and holder with xylene before regreasing to remove old grease and make a better seal. When the bottom cock is to be greased the leveling bulb is hung a little lower than this cock, the center or waste stopcock is opened and the mercury will adjust itself so that the cock can be removed. We use Cello-seal (Fisher) for the top stopcock and Peters and Van Slyke heavy grease for the bottom stopcock.

The machine may be tested for leakage by admitting two or three cc. of water into the chamber and extracting the air by

shaking. The solution is raised to the 0.5 cc. mark and the manometer read. The extraction is repeated and an increase in pressure indicates a leakage of air.[24]

Mercury is cleaned by nitric acid and water. This may be accomplished in several ways. We clean it by placing it in a suction flask with a rubber stopper and air vent and attach it to vacuum which will cause the solution to bubble through the mercury. This is then followed by distilled water and the mercury is filtered through filter paper with a small hole in it. Mercury which has been in contact with metals such as that allowed to go down the sink will form an amalgam. The mercury may be recovered by oxidation by covering it with equal parts of water and saturated sodium hydroxide and bubbling air through it for several days or as long as a week. The solution must be replaced as it becomes soiled and then the usual cleaning procedure is continued.[25]

In the original form of the machine the manometer was an open tube with a glass bulb on top and the zero point was about level with the analyst's knees. A footnote in "Quantitative Clinical Chemistry" by Peters and Van Slyke says:

> "The closed manometer really owes its origin to our laboratory comrade of many years, Glenn E. Cullen. The numerous genuflexions required during a day's work in reading the low lying zero points of the open tube told heavily on Cullen's jovial proportions, and the laboratory felt so much the loss of his usual contagious spirits, that the more humane closed tube had to be devised."[26]

The closing of the manometer removed one atmospheric pressure and brought the zero readings to a convenient level. However, the raising and lowering of the mercury may be facilitated even further by use of the handle on the leveling bulb. Strong copper wire is wound around the top of the leveling bulb and wire covered by rubber tubing attached to either side. The mercury will drain much more readily from the chamber and if many determinations are to be done, will save much time and effort.

Many medical technologists look upon the Van Slyke Neill apparatus with fear and trepidation, but with meticulous attention to a few well thought out details this machine can be one of the most valuable laboratory instruments. One of the most trying situations for the technologist arises when the machine leaks and the machine leaks, usually, because the technologist does not know how to keep it in proper condition. If one knows how to disassemble the apparatus and keeps it in good condition the Van Slyke Neill Manometric Apparatus can be put to excellent use in the laboratory with very little strain on the technologist.

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AMONG THE NEW BOOKS

PROBLEMS IN AMOEBIASIS: by Charles William Rees, Ph.D., Head, Section on Protozoal Diseases, Laboratory of Tropical Diseases, National Microbiological Institute, National Institute of Health, United States Public Health Service, Bethesda, Md., and Clarence A. Imboden, Jr., M.D. Charles C. Thomas, Publishers, Springfield, Ill. 119 pages. 18 illustrations and charts. 2 tables. $4.75.

For the investigator rather than for the clinician, there is one chapter on practical methods of diagnostic techniques. Others can be adapted. Important are notes on measures for the protection of the technologist and for the elimination of other microorganisms from fecal suspensions containing E. Histolytia.

PROBLEMS IN MICROBIOLOGY IN THE MEDICAL TECHNOLOGY CURRICULUM*

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Medical technology is one of the newest and probably the fastest growing professions associated with the field of medicine. This accounts, at least in part, for the limited amount of information available concerning methods and standards of education in the medical technology curriculum. Course work given at the university level in the departments of biology and chemistry tends to fulfill the requirements for pre-medicine and nursing, but few educators realize the needs of the medical technologist, who is neither a physician nor a nurse. Biology textbooks, for example, have been written for everyone from soil conservationist to cheese manufacturer, yet the plight of the technologist remains the same. No texts have been written for her. Students of technology at an undergraduate level struggle to comprehend course material designed for medical students, and later must rely on adequate hospital training to supply technical methods and skills.

In spite of such handicaps, medical technology is rapidly progressing. Increased enrollment in schools of medical technology in colleges and universities all over the United States bears witness to this fact.

To provide an adequate curriculum for student technologists has become the problem of many educators who recognize various needs and problems concerned with each phase of medical technology.

Since the Registry of Medical Technologists of the American Society of Clinical Pathologists has not made the study of microbiology a prerequisite for student technologists entering their hospital affiliation, it would seem more advantageous to discuss the problems related to this field, both on the college or university level and during the time assigned for its study in the clinical laboratory. It is not unusual for the technologist in charge of the bacteriology department to find a complete lack of knowledge of microbiology on the part of the student technologist. It is often discouraging for the already busy technologist to begin to cover a field which includes bacteriology, parasitology, virology, mycology and even immunology and serology. In fact, in each of these fields we find experts who have devoted their entire lives to the study of these sciences. How then is the technologist to dcide what to teach, especially since recent advances in bacterial

* Reprinted by permission from *Hospital Topics*, July, 1955.

physiology and antibiotic sensitivities have revived her department?

First of all she must consider the previous training of the student. Judging from the results compiled by a survey of universities and colleges, picked at random in the United States, the following information is available. Most universities offer a course in elementary or general bacteriology in the college of arts and sciences. Such a course would be available either to students enrolled for two years to satisfy the Registry requirements or to those enrolled in a four or five year degree program. In institutions which offer a bachelor of science degree in medical technology there seems to be a great deal of difference in the advanced courses offered and the type of subject material included in these courses. Often a course in diagnostic bacteriology is given on the junior or senior level in either the college of arts and sciences or in the school of medicine, and include anywhere from 1 to 4 (one to four) lecture hours per week and from 3 to 12 (three to twelve) hours of laboratory per week depending on the amount of credit hours the course carries in the particular curriculum. In addition to this, a course in introductory parasitology is offered in the zoology or microbiology department of the university or college.

At present the basic trend for institutions with a curriculum in Medical Technology is to offer three courses in microbiology: general bacteriology, diagnostic bacteriology and introductory parasitology. Again it must be stressed that only students enrolled in a four or five year program will be able to take advantage of these, since two year students are often advised to take a year of zoology along with either anatomy, physiology or histology to fulfill the Registry requirements. Only the exceptional two year student will enter the bacteriology department of the hospital laboratory with a general knowledge of the subject.

In addition to the basic courses offered in most colleges, some institutions have gone ahead and developed further courses in the field of microbiology. The University of California,[1] for example, requires courses in bacterial metabolism, public health bacteriology, serology, immunology and medical mycology for technologists enrolled in a five year degree program. Likewise the University of Southern California[2] includes in its curriculum additional courses in clinical and public health bacteriology, clinical protozoology, helminthology and mycology, and stress that elective credit be taken preferably in the bacteriology department. The University of Minnesota[3] offers complete courses in immunology and serology.

Larger universities throughout the Midwest have also taken great strides in the education of technologists in microbiology. Indiana University[4] includes in its course of diagnostic bacteri-

ology the theories and methods of immunology, virology and some medical mycology. The state universities of Illinois, Wisconsin and Michigan, along with Dayton and Wayne Universities, offer similar programs. In private institutions additional courses have been included both in the colleges of arts and sciences and in the schools of medicine.

The student benefits when the advanced courses in microbiology; such as immunology, serology or diagnostic bacteriology are taken in the school of medicine. The advantage seems to lie principally in the availability of clinical specimens and laboratory equipment for teaching purposes. In this regard the University of Michigan[5] provides a unique example in the method of teaching technologists from the clinical point of view. It consists in teaching bacteriology by "deduction," a term which may be defined as "teaching through knowledge of the clinical specimen." That is, the instructor gives the student a specimen of known origin and aids her in determining or "deducing" from the type of specimen what organisms may be encountered. Then the instructor proceeds to help her isolate and identify these organisms, whether they be normal or pathogenic flora.

The other method of teaching may be referred to as the "inductive" method, whereby the student acquires a theoretical knowledge of the whole field of bacteriology with little regard for the significance of the clinical laboratory specimen. With the exception of a few "unknowns" in her class work, the student knows very little about so-called "routine" clinical culture methods.

No one will deny that theoretical knowledge is essential to the student's proper understanding of bacteriology. However, institutions should also include the technical and practical aspects that will be of more value to the technologist in the medical laboratory.

It is evident that the basic problem in microbiology in the medical technology curriculum, then, is that of uniformity of instruction. Pathologists, as well as technologists in training hospitals, are well aware of their student's lack of knowledge in the field of microbiology. In most cases the technologist in charge of the department must start to teach basic concepts of bacteriology as well as laboratory technics. This is a difficult task and poses many problems to the busy technologist, who usually is responsible for the daily cultures as well as for instructing students.

To begin with, the technologist instructing in bacteriology and its allied field is faced with one or both of two unfortunate facts; one, the students do not know enough about the fundamental principles of bacteriology, and secondly, the students are not familiar with the essentials of physiology and chemistry which apply especially to bacteriology. Although educational institutions are striving for greater integration in the teaching of sci-

ences, it is too much to expect that they can teach all of the specialized phases that would be of importance to the student of microbiology. Therefore, the technologist is left to teach her subject the best she can in the few weeks allotted to its study in the laboratory.

According to the standard curriculum approved by the Registry of Medical Technologists for schools of medical technology, twelve weeks have been set aside for the study of bacteriology and parasitology. The first eight weeks are devoted almost entirely to the study of bacteriology. Sterile technics, staining, methods of culture and biochemical reactions, all of which aid in the isolation and identification of various types of organisms, must be taught. Poor staining technics and the inability to isolate organisms in pure culture are problems encountered in microbiology which should be stressed to students. So frequently students will rely on a color change in media or some insignificant biochemical reaction on which to base the identification of an organism, without understanding the reason for such changes or reactions. As a result organisms may be inaccurately identified.

Furthermore, a thorough knowledge of differential culture media is important along with knowledge of antigen-antibody reactions to confirm results. In this regard there should be close cooperation between the bacteriology and serology departments in the clinical laboratory. In order to assure such integration and correlation of bacteriological findings, many pathologists have combined the two departments with success. The student then spends sixteen weeks in the combined department, whereas he would spend twelve weeks in bacteriology and parasitology and four in serology if the departments were separately organized.

Along with routine procedures taught in the department of microbiology, the student must be taught various technical methods such as blood culture technics, pH determinations, uses of buffers, darkfield examinations and the care and uses of laboratory animals in diagnostic procedures.

The related fields of parasitology, mycology, virology and rickettsia are often neglected in the hospital laboratory. There is too much material and too little time. Then, too, instructors often lack clinical specimens. Nevertheless, the technologist has the responsibility to acquaint the student at least with basic terminology and technics. From there it is up to the student to supply herself with additional information.

Recent advances in antibiotic sensitivities and bacterial genetics are rapidly becoming involved in routine laboratory procedures. The technologist must now include concepts of bacterial metabolism, mutation rates and quantitative antibiotic assays, whereas previously she felt she had fulfilled her obliga-

tion to teach routine bacteriology.

It is true that the number of professional microbiologists is gradually increasing, but it is unlikely that they will ever meet the needs of the clinical laboratories. Medical technologists will continue to take the responsibility of the microbiology department in the hospitals, clinics and doctors' offices.

It is evident that problems exist, and it will be up to the profession and its members to help solve them. Prospective students should be advised to take at least general bacteriology in college. Plans should be considered and eventually proposed to the Registry of Medical Technologists to add bacteriology to its list of advised biology courses instead of merely adding it as a recommended course. A knowledge of zoology, anatomy and physiology is extremely important. Nevertheless, bacteriology is equally so, along with hematology and clinical pathology; the latter is usually available only to upperclassmen in the universities and not to two year students of technology.

With increasing enrollment of student technologists, universities and colleges will continue to include additional course work in the field of microbiology. There is hope that in the future there will be close cooperation between educational institutions and training schools, the universities supplying as best they can the didactic knowledge of the basic sciences, and the hospitals providing the practical and technical methods of laboratory procedures. The realization of this hope will mean inevitable progress for medical technology.

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FROM OUR READERS

Hints: From Dr. F. H. Connell (formerly from New Hampshire), M. D. Anderson Hospital, Houston, Texas, who says, "Try using plastic silverware trays—the kind with 4 or so compartments—for dirty blood count pipettes. This keeps them separated—saves breakage, too." Have you adapted any other household plastic ware to the lab?

PAPER ELECTROPHORESIS*

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Paper electrophoresis is a relatively new addition to the armamentarium of the protein chemist. The scant seven years since the discovery of this technique for protein analysis have witnessed a phenomenal growth in its popularity. The use of paper electrophoresis is today almost entirely limited to the research laboratory, but it is now ready for the clinical laboratory. The simplicity, accuracy and low cost of the technique make it quite desirable.

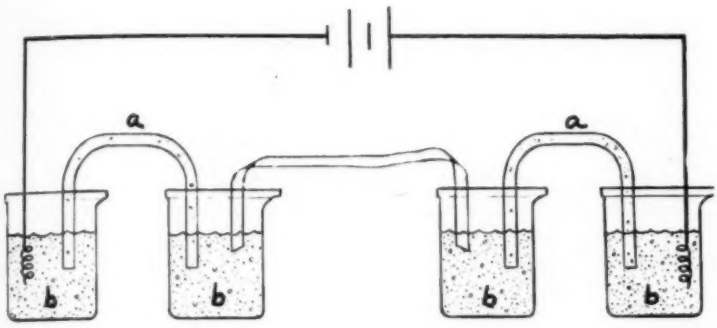
Electrophoretic analysis depends in principle on the migration of charged particles in an electric field. Proteins in proper media are charged particles, and their migration in an electric field provides a qualitative and quantitative method of analysis.

The technique of paper electrophoresis is briefly, as follows: A piece of filter paper is saturated with buffer solution and suspended between two containers of the same buffer solution with each end of the paper dipping into the buffer containers (fig. 1). A salt bridge or baffle system is interposed between the electrodes and the buffer container into which the paper is dipping. This isolates the paper from the intense pH changes which occur about the electrodes. A quantity of blood serum or plasma is placed on the paper and direct electric current is applied across the paper through the buffer solutions. The protein in the serum migrates in the electric field at a rate proportional to the charge on the protein. The charge on the protein is a function of the hydrogen ion concentration of the buffer used due to the amphoteric property of the amino acids constituting the protein.

Figure two illustrates a normal serum protein pattern. The point at which the serum was applied to the paper is marked with a line. The graph above the filter paper strip is drawn from optical density measurements of the paper strip, and is plotted as optical density versus distance of migration. The greatest single concentration of protein lies to the anodal side of the loading point; this is albumin. The other zones from the albumin toward the cathodal end of the strip in order are the alpha 1, alpha 2, beta, and gamma globulins.

Those factors which influence the rate, sharpness, and degree

* Read before ASMT Convention, June, 1955, New Orleans, La.



PAPER ELECTROPHORESIS

Fig. 1. Schematic diagram showing the relationships in paper electrophoresis. (a) agar bridge, (b) buffer solution and the paper strip is suspended between the two inner buffer containers.

of separation of the protein constituents may now be considered. First, a source of direct current is needed. A transformer and rectifier for converting the ordinary alternating house current to direct current is used. There is today a controversy as to whether this rectifier should deliver a constant current or constant voltage. Suffice it to say here that each has its advantages and disadvantages. The most commonly used power supply is one delivering a constant voltage. Secondly, this current must be applied across the paper strip. Electrodes in an electrolyte produce electrolysis when the current flows. The electrolyte used in this system contains sodium which in solution exists as positively charged sodium ions. The sodium ion will migrate to the negative electrode where it accepts an electron to become sodium metal. This immediately reacts with the water producing sodium hydroxide. Thus about the cathode there will be an intense change in hydrogen ion concentration, making it necessary to isolate the paper strip from the electrode by some means. Similar changes occur about the positive electrode; where the negative ions lose an electron. Two methods are available to accomplish this—a salt bridge such as is illustrated in figure one or a series of baffles.

The electrolyte solution used to conduct the current across the paper strip must be a buffer solution if the protein surface charge is to remain constant and thus yield predictable migration. There is no controversy about this buffer; a solution of barbituric acid the sodium barbital at pH 8.6 is in almost universal use.

The conduction of a current by any solution depends upon

the number of charged particles (ions) available to carry the current, or otherwise stated the concentration of the solution with regard to ionized particles. The more ions in solution the greater the ease with which the solution can conduct a current; or in electrical phraseology, the less will be the resistance of the solution. The flow of an electric current across a resistance results in the production of heat. This heat causes water to distill from the paper strip which produces two undesirable ends. First, the loss of water from the paper increases the concentration of solute in the paper and thus lowers the resistance of the paper to a current flow. This alters the rate of migration of the protein especially at the edges of the paper where the water loss is greatest and produces a distortion of the moving boundary referred to as "edge effect." The second effect produced by water loss from the paper is the migration of fluid from the buffer containers at the ends of the paper toward the areas of greater solute concentration in the paper. This migration alters the moving protein boundary.

A number of methods are available to minimize this distillation of water from the paper strip. The strip may be compressed between two plates thus excluding air. Enclosure of the system in a container of very high humidity greatly reduces distillation. The differential water loss at the edges of the paper strips in an apparatus handling multiple paper strips may be minimized by placing the individual strips as close together as possible. The addition of glycerine to the buffer solution reduces the buffer vapor pressure without an appreciable effect on conductivity.

The perfect paper for electrophoresis has yet to be found. A paper of absolutely uniform porosity would be the perfect one since innumerable pores in paper attract fluid by capillary attraction. Another effect produced by the paper is electroosmosis. The cellulose in paper has a small number of free carboxyl groups possessing a negative charge, which produces a liquid flow in the direction of the positive current, and may cause negatively charged proteins to move in the wrong direction. Since this is constant in direction and magnitude is causes no difficulty in protein pattern interpretation provided its existance is appreciated.

The technique for electrophoretic analysis as used in this laboratory is presented for purpose of illustration. A sample of blood is collected and the serum separated. The buffer tanks are filled with a barbital buffer of pH 8.6 and molar strength of 0.05. This particular molar strength is used, because with the available apparatus, experience has shown that this has the proper conductivity to give sharp protein separation at the desired voltage and time. The apparatus has a large center well which is filled with water so that when a cover is applied the

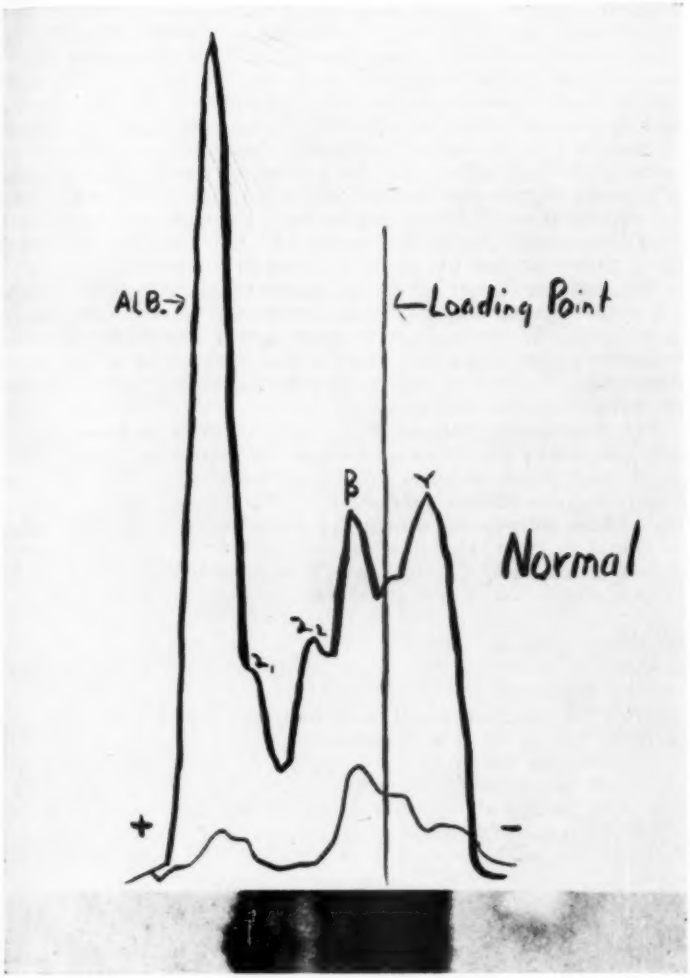


Fig. 2. Serum Protein curve derived by plotting the optical density values against the migration from the loading point. The curve is for the normal serum on the strip below.

humidity inside will be as nearly saturated as possible. The paper strips are moistened in the barbital buffer, blotted with paper towels until the liquid sheen just disappears, and placed between the buffer containers with one end dipping into each. Before the protein is applied the apparatus is tightly covered and the current turned to 200 volts. The number of milliamperes of current that flows is governed by the ionic strength of the buffer and the length and width of the paper strips. The current is allowed to flow for one-half to one hour, allowing ample time for equilibration of buffer migration due to capillary attraction, electrical forces (electro-osmosis), and gravity. The current is then turned off and the serum applied to the paper strips.

The protein is applied to the paper strips with a ten lamba (0.01 ml) pipette. The use of an accurate pipette is only necessary when the results are to be assessed quantitatively. The cover is again closed and the current applied for a period of four hours. At the end of this time, the current is turned off and the paper removed and air dried.

The dried paper strips on which the protein has been separated may be stained much like a histologic section. This is one of the great advantages of paper electrophoresis. Conventional free electrophoresis allows estimation of the whole protein but not the various substances which are bound to protein. The paper strip may be stained for total protein, protein bound lipid, protein bound carbohydrate, and an ever increasing list of other protein bound substances. The most commonly used protein stains are bromphenol blue and amidoschwarz 10B. The stain most used for demonstrating protein bound lipid is oil red O. The demonstration of protein bound carbohydrate utilizes a modification of the periodic acid Schiff reaction.

Paper Electrophoresis is, in addition, an invaluable aid in the study of certain hemoglobin abnormalities. Particular care must be exercised in the preparation of the red blood cell hemolysate since the specimen when ready for electrophoresis must be as free from plasma and red blood cell stroma proteins as possible. These proteins when present may be responsible for "smudge" patterns and may give confusing background if the strips are stained.

The technique for preparation of the hemolysate is as follows: Three to five ml of oxalated or heparinized blood is centrifuged in a 15 ml centrifuge tube for 10 minutes at 1500 rpm and the supernatant plasma discarded. The cells remaining are washed with normal saline 4 or 5 times, or until the supernatant shows no turbidity to the addition of 3% sulfosalicylic acid. The packed cells are then frozen to lyse the cells. Following thaw and equal volume of 0.05 M barbital buffer (same as when used for protein) and ½ volume of toluene is added to the same tube.

The mixture is thoroughly shaken and refrigerated overnight. Following refrigeration the mixture is centrifuged at 2000 rpm to aid in separation of the toluene-red cell stroma protein layer from the hemoglobin. The pink creamy red cell stroma protein layer is removed, discarded, and the remaining hemolysate is filtered through a close mesh filter paper, (Whatman #5 or equivalent). The hemoglobin concentration of the filtrate is adjusted to approximately 10 g% by the addition of buffer solution. This hemolysate should be stored in the frozen state for maximum life. Determination of the fetal (F) hemoglobin by the alkaline denaturation technique of Singer, (1) may be performed on this hemolysate.

The hemoglobin solution prepared in the above manner is loaded and run in exactly the same manner as that used for serum or plasma proteins, except that the time of migration is increased to 6 to 8 hours. Because of the prolonged period of migration and the various effects of distillation from the strips addition of glycerine to a concentration of 2% in the buffer adds considerably to the perfection of the separation. Control specimens of known hemoglobin types must be run simultaneously with all unknown specimens. Figure three shows separation of A (normal adult), S (sickle) and C hemoglobins.

The amount of actual working time required for a paper elec-

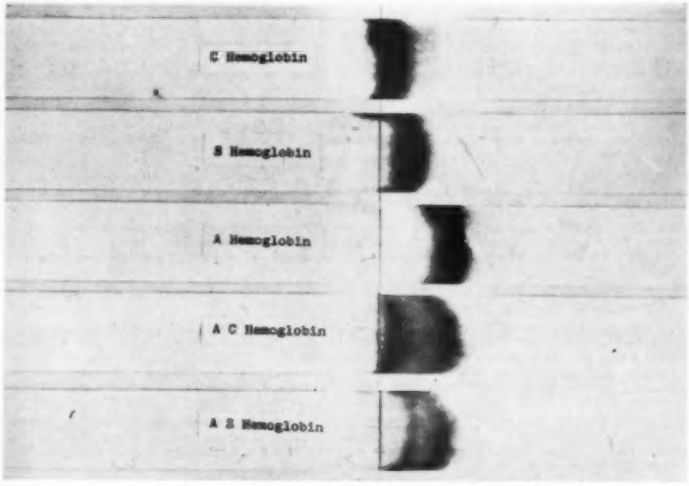


Fig. 3. Separation of hemoglobins. Note that hemoglobin C stays closest to the loading point with S (sickle) next and A (adult) migrating the fartherest.

trophoretic analysis is less than one hour and multiple analyses may be accomplished within the same amount of time. The salt fractionation method of albumin and globulin concentration requires at least this much time and the values obtained do not represent the actual concentration. The albumin figure obtained by some salt fractionation techniques includes both alpha globulin and a portion of the beta globulin plus albumin.

To the clinician protein analysis is an aid to diagnosis and to clinically follow a wide variety of diseases. Some of those clinical conditions in which protein fractionation may be an aid are liver diseases, multiple myeloma, connective tissue diseases, infections, and renal disease. This technique establishes a positive diagnosis in agammaglobulinemia and some hemoglobin disorders.

Paper electrophoresis gives an accurate protein fractionation with a minimum of technologist time. The simplicity of the technique coupled with the accuracy of protein fractionation make it very useful for the clinical laboratory.

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BACILLARY DYSENTERY WITH BACTEREMIA*

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This paper is presented to report the unusual condition of shigellosis with bacteremia. From two patients with bacillary dysentery seen at Charity Hospital, Shigella flexneri was isolated from the blood of one and Shigella sonnei from the blood of the other. These are the only two such occurrences in 60,680 blood cultures made during the past 10 years at our hospital. The unusualness of this finding led us to survey the literature concerning Shigella bacteremia.

Zinsser's Textbook of Bacteriology[14] states that in bacillary dysentery the organisms may sometimes reach the lymph nodes but rarely, if ever, invade the blood stream. A similar idea is expressed by several standard textbooks of bacteriology.[5, 11, 17] In surveys made for the specific purpose of attempting to isolate Shigella from the blood of patients with bacillary dysentery positive cultures were very rare. Kessel[12] in 60 and Dudgeon[6] in 145 blood cultures reported negative results, while Frankel[9] obtained one positive culture from 49 blood specimens. 38,000 cases of shigellosis were reported in this country during 1944, but there is not a reported case of Shigella bacteremia during this period.

There are, however, approximately 37 cases of Shigella bacteremia on record from 1900 to 1953,[2, 8, 15] and this, together with the fact that they have been reported isolated from the mesenteric glands, liver, and spleen at autopsy,[3, 7, 8] indicates that bacteremia may be more common than is usually supposed. In the cases of bacteremia the responsible agents were members of the Shigella flexneri and dysenteriae groups in about the ratio of 2/1. The first and only case of Shigella sonnei was reported in 1951. The present report is the second such case, while adding another case to the group of Shigella flexneri bacteremias.

Report of Cases

Clinical Data

Case #1. C. M., an 18-month-old Negro infant, was admitted to Charity Hospital on 10-19-53 with symptoms of high fever and diarrhea for one day. On the day of admission he had had numerous loose stools described as watery, green, foul smelling, containing much mucus but no blood. He had had fever with one generalized clonic convulsion of several minutes' duration and an upper respiratory infection for several days before the

* Third Award, 1955, Scientific Products Foundation, Bacteriology Division. Read before ASMT, June, 1955, New Orleans, La.

onset of fever and diarrhea. In June, 1952, he was hospitalized for 2 days at Charity Hospital for a short generalized convulsion and fever, temperature 103 F. No cause was found for these symptoms which promptly subsided. The past history is otherwise negative except that he was a premature infant.

On admission the temperature was 104.4 F. rectally, pulse rate 160 and the child seemed drowsy. The remainder of the physical findings were essentially negative.

On admission the hematocrit reading was 30 and the leukocyte count 8,800 with 88 per cent neutrophils and 12 per cent lymphocytes. A serological test for syphilis and febrile agglutinations for typhoid, paratyphoid and typhus fever, and brucellosis were negative. Microscopic and chemical examination of the cerebrospinal fluid and urine was negative. Alpha hemolytic streptoccus and Staphylococcus aureus pyogenes were cultured from the throat. The blood culture was positive for Shigella sonnei. The stool was negative for pathogens at that time, but Shigella sonnei was isolated two days after admission. The stool was negative for pathogens 10 days after admission and on 3 subsequent examinations.

The antibiotic and chemotherapy consisted of sulfadiazine, penicillin and oxytetracycline.

The child showed prompt clinical improvement. The temperature present on admission was normal on the second day, and the diarrhea cleared by the third day. He was discharged 2 weeks after admission well and was seen one week later in the clinic in excellent condition.

Case #2. C. C., a 1-month-old Negro infant, was admitted to Charity Hospital on 10-8-53 for severe diarrhea of 2 days duration. The stools were described as watery, green, with scant mucus but not foul smelling. The child had had an upper respiratory infection for several days prior to the onset of diarrhea. The past history was negative.

The physical examination on admission disclosed an acutely ill, badly dehydrated infant. Respiratory distress was present and cyanosis of the face and extremities was noted. The neck was held in marked extension, but was not stiff. Coarse inspiratory rales were heard in the base of the right lung posteriorly. The temperature was 100 F. rectally and the pulse rate 180.

On admission the hemoglobin was 10.2 grams and the leucocyte count 9,750, with 27 per cent neutrophils and 73 per cent lymphocytes. The urine was negative. The carbon dioxide combining power was 29 volumes per cent. The cerebrospinal fluid was clear and colorless, but contained 60 lymphocytes and 62 mg. protein, 54 mg. glucose and 670 mg. chloride per 100 ml. A culture of this fluid for bacteria was negative. A culture of a nasal swab was positive for Staphylococcus aureus pyogenes, Sta-

phylococcus albus saprophyticus, Diptheroids and Alcaligenes. A stool and blood culture were positive for Shigella flexneri. Two stool cultures were negative for pathogens immediately prior to discharge.

The infant was placed in an oxygen tent and the dehydration and acidosis were treated with parenteral fluids.

Antibiotic and chemotherapy consisted of sulfadiazine, penicillin and chloramphenicol.

The child was critically ill the first day, but on the second day began to show marked clinical improvement, at which time semi-solid stools were present which became formed on the third day. No significant degree of fever was present during the course of the illness. She was discharged on the fourteenth day, and was well when seen in the clinic 1 week later.

Bacteriological Findings

Case # 1. A blood culture drawn on admission was inoculated into brain heart infusion with 0.1 per cent agar and from this a pure culture of non-motile, gram-negative rods was obtained after 48 hours' incubation. These organisms produced acid with no gas in dextrose and mannite. After 96 hours' incubation acid was produced in lactose. There was no acid produced in sucrose, xylose or rhamnose. Indol, urea and citrate utilization tests were negative. These organisms were agglutinated with Lederle's Shigella Group D (sonnei) antisera. The same type of organism was isolated from the stool two days after admission.

Case #2. A blood culture drawn on admission was inoculated into brain heart infusion with 0.1 per cent agar and from this a pure culture of non-motile, gram-negative rads was obtained after 48 hours' incubation. These organisms produced acid with no gas in dextrose, mannite and rhamnose. No acid was produced in lactose, sucrose and xylose. Ehrlich's test for indole was weakly positive. The organism did not utilize citrate or urea. A saline suspension of the organism agglutinated Lederle's antisera for Shigella Group B (flexneri). The same type of organism was isolated from a stool taken on admission.

Discussion

The bacteriologic findings are not unusual and will cause no further comment except to state that the organisms were isolated without difficulty in our standard blood culture medium which consists of Difco Brain Heart Infusion with 0.1% agar.

The significance of Shigella bacteremia is not known. There is apparently no clinical correlation between the presence of Shigella organisms in the blood stream and the severity of the illness. Bacteremia has occurred in both mild and severe cases with complete recovery, and in fulminating cases in which death occurred.[4, 8, 10, 13]

Most, but not all, of the cases have had dysentery. Of the

reported cases, as near as can be determined, 11 deaths have occurred, but of these cases which were autopsied, no lesions were present which are not found in patients dying without sepsis.[4, 8, 10, 13]

In those cases of sepsis in which death occurred the duration of the illness was usually a few days,[4, 8, 10, 13] not exceeding 12, with the exception of the case in which death occurred in the 10th week.[2] The most fulminating type of bacillary dysentery resulting in death within 48 hours, usually revealed no bacteremia.[8] The bacteremia produces no characteristic clinical picture, although several cases have been reported resembling typhoid fever.[2, 8, 13] Rothman,[13] in his review, cites a case of arthritis, meningeal involvement, pneumonia, thrombophlebitis, and acute nephritis associated with sepsis as evidence that sepsis apparently favors complications.

The stage at which the organisms enter the blood stream is not known. In most of the reported cases the organisms were cultured from the blood during the first few days of the illness.[4, 8, 10, 13] Notable exceptions to this are the cases in which an organism was isolated from the blood on the 23rd day and in another case in the 7th week[2] of the illness.

In mild, severe and even fatal infections positive blood cultures for shigellae in the absence of positive fecal cultures have been noted.[8, 13]

The rarity of reported cases of Shigella bacteremia may be due to the fact that blood cultures are not usually made to aid in the diagnosis of dysentery, or the blood stream may become sterile rapidly following the entrance of the organisms as is the case of the experimental animal.[8] Only one case has been reported with more than one positive blood culture, it having two.[16] Certainly, the lesions[1] produced in the intestines by these organisms affords an adequate opportunity for the entrance into the blood stream.

The treatment with sulfadiazine, chloramphenicol, and oxytetracycline followed the recommended chemotherapy.[14]

The presence of Shigella in the blood stream in one mild case and one moderately severe case of bacillary dysentery with prompt clinical recovery is in keeping with similar cases previously reported in the literature.

Summary

Two cases of bacillary dysentery with bacteremia are reported. One is apparently the second recorded case of a Shigella sonnei bacteremia, and the other adds a case to the small group of Shigella flexneri bacteremias.

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THE GAVEL

"With ever increasing strength and humility and with the pride and integrity of our profession, LET US SERVE," is inscribed on our gavel. This ideal is an inspiration to those whom you have chosen to guide your professional society. Another milestone has been added to the American Society of Medical Technologists with the coming of the end of this fiscal year. I appreciate this opportunity to thank the officers, members of the Board of Directors, Board of Schools, and all committee members for their sincerity and faithfulness in carrying out their assigned duties. What has been accomplished this year, we owe to them. The June NEWS RELEASE has their annual reports for your information. The Editor of the American Journal of Medical Technology has done a wonderful job of editing, and your journal has grown, not only in size, but in stature too. With great anticipation we look to the First North American Conference of Medical Laboratory Technologists for the grand finale. So we must say "thanks" again, and our very best wishes for the future of the American Society of Medical Technologists.

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METHOD FOR QUALITATIVE AND QUANTITATIVE STUDIES OF FECAL FLORA*

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Bacteriological studies of fecal flora have become increasingly important since the advent of broad-spectrum antibiotics. These studies are usually carried out with three aims: (1) to find the best antibiotic or the best combination of antibiotics to be used for intestinal antisepsis; (2) to study the overgrowth of certain microorganisms as a complication of the antibiotic therapy and (3) to estimate the degree of intestinal implantation of certain flora employed as a possible therapeutic measure. In any of these cases it is essential that the method of study should be time-saving and yet accurate enough to enumerate and identify the different groups of the flora. The following method is presented and discussed in accordance with this premise.

Handling of Specimens

The fecal specimens are collected in clean wax-coated cardboard containers such as half-pint ice cream containers and should not be contaminated with urine. The specimens should be studied immediately or as soon as possible. If delay is necessary, the containers must be sealed with adhesive tapes and the specimens immediately refrigerated. A specimen left for more than 2 days even in a refrigerator should not be deemed satisfactory. The gross characteristics of each specimen should be first noted and recorded. To prepare fecal suspension a sample totaling one gram is taken from several portions of each fecal specimen and is then emulsified in 9.0 ml of sterile diluent in a large test tube (150x18mm) representing a sample of 10^{-1} dilution. Either distilled water or physiological saline may be used as the diluent since similar results are given by the two diluents as illustrated in Table I. The mixture is then blended into a homogenous suspension with sterile wooden applicators or glass rods and finally filtered through two layers of gauze

TABLE I

Effect of Diluents on Proteus Counts in Feces (Loh, 1952)

DILUENTS	Dilution of Feces	Average No. of Colonies Counted on Duplicate Plates	No. of Organisms/ gm. of Feces
Physiological saline	10^{-6}	119	1.2×10^8
Dist. water	10^{-6}	106	1.1×10^8

Remark: Both series of dilutions were prepared from the same 10^{-1} suspension of feces. SS plates and surface streaking technic were used.

* Received for publication October, 1955.

over a sterile funnel. Some investigators prefer a flask to a test tube and emulsify the fecal sample with glass beads inside the flask and the aid of an electric shaker. The sample of 10^{-1} dilution can also be prepared from the entire felcal specimen to which a calculated volume (9 times its weight) of distilled water is added. The mixture can then be blended into a homogenous suspension in a Waring blender. In the writers' experience, the suspension prepared from one-gram sample is just as satisfactory as that prepared from the entire specimen. The 10-ml sample of 10^{-1} dilution is then diluted in sterile distilled water into a series of 10-fold dilutions (10^{-2}, 10^{-3}, 10^{-4},—etc.) according to the technic approved by the A.P.H.A. for water analysis (1). The tubes (150x18mm) used for the serial dilutions of the sample should be sterile, cotton-plugged and should each contain 9.0 ml of sterile distilled water. It is suggested that before the 1-ml sample is transferred from one tube into another tube of higher dilution the former tube should be shaken 25 times to ensure an even distribution of the fecal flora. Generally, sterile 1-ml serological pipets graduated at 0.1 ml are very satisfactory for transferring the sample as well as for inoculating the sample to plates with surface streaking technic.

Organisms To Be Studied, Mediums of Choice and Methods of Study

A. *Total aerobes:* They can be very satisfactorily enumerated in pour plates of B.B.L. Eugonagar using a 1.0-ml inoculum for each plate. The medium is enriched, being particularly good for fastidious organisms to grow. In making pour plates, not more than 20 minutes should elapse between the preparation of dilutions and the final pouring of plates.

B. *Total anaerobes (including facultatives):* Brewer anaerobic culture dishes containing B.B.L. anaerobic agar have been found satisfactory for the cultivation and enumeration of these organisms. Precautions in using the dishes and preparing the medium are given in the literature provided by the Baltimore Biological Laboratory, Inc. To achieve satisfactory results the medium in the dishes should be freshly prepared and its surface devoid of excessive condensation of moisture. It is advisable to pour the melted medium into clay-top dishes to cut down moisture on surface of the medium and then replace the clay tops with the Brewer anaerobic tops after allowing the dishes to stand for 6 to 8 hours. 0.1-ml inoculum per plate is used for surface streaking. The surface streaking can be easily performed by moving the tip of the pipet gently back and forth over the area to be inoculated while the plate is kept rotating with left hand. The pipet should have smooth ends and is held at an angle of 45 degree controlling the smooth delivery of the inoculum with

the right index finger over the upper end of the pipet. With this technic it is not necessary to spread over the medium with a bent glass rod.

To enumerate spore-forming organisms the sample for inoculation should be first subjected to heating at 80° C for 10 to 15 minutes. If an anaerobic jar is available, one can use pour plates of blood agar to enumerate the total anaerobes. *Clostridium perfrigens* usually present hemolytic and triangular subsurface colonies. Their further identification requires Litmus milk medium. In the writers' experience, the egg-yolk medium of McClung and Toabe (2) for presumptive identification of lecithinase-producing clostridia did not prove satisfactory. Fluid or solid thioglycollate medium were satisfactorily used by some investigators to enumerate the total anaerobes. The modified Wilson Blair Medium for clostridia (3) appears to be an excellent medium for pour plates.

Bacterioides are strictly anaerobic and fastidious organisms requiring 4 to 7 days to grow at 37° C. Their enumeration and identification are usually excluded, although they actually constitute a great bulk of the fecal flora.

C. *Coliforms:* The coliforms can be satisfactorily enumerated in Difco Violet Red Bile Agar using a 0.5 ml or 1.0 ml inoculum for making pour plates. The superiority of this medium over many other mediums for lactose-fermenters was previously confirmed by Yale (4) in 1937. After 15 to 24 hours' incubation at 37° C typical coliform colonies are deep red, 1 to 2 mm in diameter, surrounded by a reddish zone of precipitated bile salts. Further identification of the coliforms requires MR-VP, indol, citrate and fermentation tests. Difco Tergitol-7 Agar with added TTC (triphenyltetrazolium chloride) has the advantage to provide presumptive differentiation between *Escherichia* and *Aerobacter* on plate.

D. *Yeasts:* Yeasts can be initially isolated and enumerated on grape juice agar using 0.2 ml inoculum for surface streaking. The grape juice agar is easily prepared by suspending 40 gms of Difco Bacto-Agar in 750 ml of distilled water to which 250 ml of commercial grape juice (First National Stores) has been added. This is then mixed thoroughly and boiled gently for 3 to 5 minutes, cooled and poured into Petri dishes. The medium contains approximately 5 per cent of sugar and 4 per cent of agar, and has a final pH about 3.9. In the writers' experience, it exerts no inhibition on the growth of yeasts and appears to be very selective, allowing only the growth of yeasts and other fungi probably due to its low pH. *Candida albicans* on this medium produce white creamy circular convex entire colonies. Other fungi produce colonies of different contour and color ranging from pink to light purple. *C. albicans* can be further confirmed

by its ability to produce pseudomycelia and chlamydospores at room temperature in corn meal agar (5) or in Difco Chlamydospore Agar or in zein agar described by Reid, Jones and Carter (6). The zein agar provides a more rapid and profuse production of the chlamydospores.

E. *Staphylococci:* They can be satisfactorily isolated and enumerated on Difco Staphylococcus Medium using 0.2 ml inoculum for surface streaking. The medium contains 7.5 per cent sodium chloride to allow a fairly selective growth of the staphylococci. Pigment production of the organisms on this medium can be clearly detected. Some investigators prefer blood agar to this medium for the identification of staphylococci. The blood agar has the advantage in bringing out hemolytic property of the organisms but, on the other hand, has a great disadvantage of nonselectiveness thus allowing *Proteus* to swarm. The rapid growth of *Proteus* is frequently encountered following administration of broad-spectrum antibiotics. The organisms so obtained on the Difco Staphylococcus Medium should be further confirmed with Gram stain and catalase test which can be easily carried out by applying one loopful hydrogen peroxide solution on the colony to produce air bubbles in case of positive catalase test. Representative strains of *Staphylococcus* aureus should be further tested for its pathogenicity with coagulase test. Dearing and Heilman (7) described their satisfactory use of B.B.L. Phenylethyl Alcohol Medium to detect the presence of staphylococci. Phenylethyl alcohol inhibits growth of gram-negative bacteria; medium containing it was said to be particularly useful for the growth of staphylococci in the presence of *Proteus.* It is the feeling of the writers that this medium has the disadvantage in exerting some inhibition on the growth of the staphylococci themselves.

F. *Streptococcus faecalis (enterococci):* They can be satisfactorily enumerated using Difco S F Medium containing sodium azide. Five tubes of the liquid medium are inoculated with each appropriate dilution and incubated at 45.5° C for 48 hours. When no growth occurs in any of the tubes after two days' incubation, the tubes should be re-incubated for an additional 24 hours. From the number of positive tubes, the most probable number (MPN) of *Str. faecalis* in each specimen can be determined from the tables of Prescott and co-workers (8). It should be pointed out that tubes of low dilutions (10^{-1}, 10^{-2}) showing turbidity but no significant change of color after incubation should not be considered positive, because the turbidity could come easily from the addition of heavy fecal inocula. The positive tubes should be occasionally checked with Gram stain for purity of the cultures. For plate counting Chapman's Streptococcus Medium (9)

TABLE II
Identification of Proteus

SPECIES	FERMENTATION OF					Prod. of		Urea Reaction
	Glucose	Lact.	Mannitol	Maltose	Sucrose	Indol	H_2S	
P. vulgaris	AG	—	—	AG	AG	+	+	rapid
P. mirabilis	AG	—	—	—	AG (late or neg.)	—	+	rapid
P. morganii	AG	—	—	—	—	+	—	slow
P. rettgeri*	A	—	A	—	A (late or neg.)	+	±	rapid

* This species should be excluded from the genus **Proteus**, because of its inability to produce amines.
A=acid; G=gas.

for surface streaking has been satisfactorily used by some investigators (10).

G. *Proteus, Paracolon bacilli and Pseudomonas:* They have been isolated and enumerated on Difco S S Medium using 0.2 ml inoculum for surface streaking by the writer (11, 12, 13). *Proteus* produce colorless and somewhat flat colonies frequently having black centers and are unable to swarm on the S S agar. The species of *Proteus* can be determined from Table II modified after Proom and Woiwood (14). The writers have not been successful in the attempt to use the urea ricinoleate agar (15) for the enumeration of *Proteus* in feces. *Paracolon bacilli* usually produce light pink colonies frequently having black centers on the S S agar. The pink color usually becomes deeper upon incubation for a longer period of time. Representative strains should be further specifically identified culturally. Although *Pseudomonas* can be recognized on the S S agar by the presence of some evidence for their characteristic pigment production, a selective medium for this organism is sometimes desirable. Very recently, Lowbury and Collins (16) described a promising selective medium containing 0.03 per cent of a new cetrimide product and a phosphate buffer at pH 7.8 for *Pseudomonas pyocyanea.* As the new cetrimide is still not a pure compound, new batches should be individually tested to establish the optimal concentration of the cetrimide which is critical for the selective nature of the medium. For better demonstration of pigment production two simple mediums were recently described by King, Ward and Raney (17).

H. *Lactobacillus acidophilus:* The isolation and enumeration of this organism from feces was recently studied on four kind of medium—Difco Tomato Juice Agar, Difco Trypsin Digest Agar, Ficin Digest Agar and B.B.L. Lactobacillus-Selection Medium (LBS Medium). The former three are roughly comparable with the possibility that Ficin Digest Agar might be slightly superior to the other two. The remaining LBS Medium has the advantage in suppressing the growth of other organisms but also has a great disadvantage in suppressing to certain extent the growth

of *Lactobacilli* themselves requiring a much longer period of incubation. The experimental data of this study are summarized in Table III. Any of the former three mediums should prove satisfactory for the study of this organism. The combined use of LBS Medium may sometimes help to isolate the organisms especially from heavy fecal inoculum. For any of these mediums the plate is usually inoculated with 0.2 ml inoculum for surface streaking. After 10 to 20 minutes of standing, the inoculated surface is then overlaid with a thin layer of Eugonagar to enhance the growth of *Lactobacilli* under reduced oxygen tension. Typical subsurface colonies of *L. acidophilus* are recognized by their dense centers and fuzzy extremities. Yeasts and occasionally *Streptococci* in the feces are the chief offenders that grow rapidly in any of the mediums. Representative colonies should be further studied with Gram stain and sometimes with fermentation and coagulation tests too.

The medium of choice and method of inoculation for each group of organisms are summarized in Table IV. All the plates aforementioned for the various groups of organisms are incubated aerobically at 37° C for 48 hours, followed by 24 hours' incubation at room temperature before final counting. The incu-

TABLE III

Comparison of Mediums for Cultivation and Enumeration of Lactobacillus acidophilus (Loh, 1955)

MEDIUM		Tomato Juice Agar	Tomato Juice Agar (Difco)	Trypsin Digest Agar	Trypsin Digest Agar (Difco)	Lactobacillus—Selection Medium	Lactobacillus—Selection Medium (B.B.L.)	Ficin Digest Agar*	Ficin Digest Agar*
		S	FS	S	FS	S	FS	S	FS
Average Number of Colonies per Plate	48 hours' incubation	82	14	80	14	17	12	87	16
	96 hours' incubation	84	16	80	21	30	18	88	24

S = Lactobacilli in saline
FS = Lactobacilli added to fecal suspension (10^{-9})
} containing same amounts of *L. acidophilus*

All the plates are incubated aerobically at 37° C.
Each of the values given in the above table represents an average of the numbers of colonies counted on duplicate plates.

* The Ficin Digest Agar consists of:

Ficin digest	500 ml.	autoclaved for 15 minutes at 15 pounds steam pressure
Distilled water	400 ml.	
Tomato extract	100 ml.	
Granulated agar	20 gms.	

The ficin digest is made by incubating for 2 hours at 45° C.:

Skim milk powder	50 gms.
Ficin enzyme (Merck)	5 gms.
Distilled water	500 ml.

The above mixture is acidified to pH 4.5-4.7, brought to boiling and filtered. The filtrate is neutralized to approximately a pH of 6.8, autoclaved for 10 minutes at 15 pounds and filtered again. This filtrate is the ficin digest referred to above.
The tomato extract is made by bringing a mixture of equal parts of water and canned whole tomatoes which have been crushed by hand to boiling and then filtering. The filtrate is the tomato extract.

TABLE IV
Medium of Choice and Method of Inoculation

Organism	Medium of Choice	Method of Inoculation
Total aerobes.....	BBL Eugon agar	Pour plates
Total anaerobes...	BBL anaerobic agar	Surface plates
Clostridia.....	Modified Wilson Blair Medium	Pour plates
Coliforms.....	Difco violet red bile agar	Pour plates
	(Difco Tergitol—7 agar)	Surface plates
Yeasts.....	Grape juice agar	Surface plates
Staphylococci.....	Difco staphylococcus medium	Surface plates
	(BBL phenylethyl alcohol medium)	Surface plates
Streptococci.....	Difco S F medium (Chapman's streptococcus medium)	Surface plates
Proteus.....	Difco SS medium	Surface plates
Pseudomonas.....	(Difco SS medium); Cetrimide medium	Surface plates
Paracolon bacilli..	Difco SS medium	Surface plates
Lactobacillus	Ficin digest agar	Surface plates*
acidophilus.....	(BBL LBS medium)	Surface plates*

* Overlaid with a thin layer of Eugone agar.
Mediums in parentheses are mediums of second choice.

bation at room temperature could especially help the *Staphylococci* for better production of pigments. The coliforms are usually counted at the end of the first 24 hours' incubation and the total anaerobes enumerated at the end of 48 hours' incubation. All the plates mentioned above are made in duplicate.

Counting and Graphic Presentation

The final count for each kind of organisms represents an average of the numbers of colonies counted on duplicate plates.

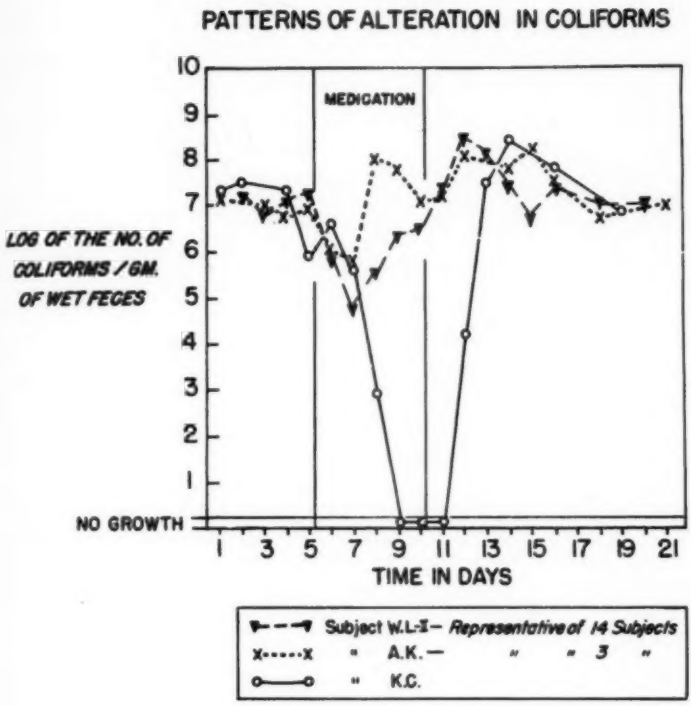
TABLE V
Precision of Plate Count (pour plates)

P = plating error (approximately ±10 percent)
D = dilution error (approximately ±2.8 percent for each stage of dilution)
If a 4-stage dilution is used then total error of the plate counts,

$$\text{"E"} = \pm \sqrt{P^2 + D^2} = \pm \sqrt{(10.0)^2 + (4 \times 2.8)^2} = \pm 15.0\%$$

The counting may occasionally require the aid of a Quebec colony counter or a hand magnifying lens, especially when the colonies are too small to be identified with naked eye. Each chain of ill-divided colonies should be counted as a single colony, for each is probably the result of fortuitous disintegration of a group of organisms caused by the mixing of inoculum and medium. A plate giving the most reliable count should have colonies between 30 and 300. Each count is then converted into the number of organism per gram of wet feces by taking the size of inoculum and the dilution factors into consideration. This is finally converted into log number of the organism per gram of wet feces before entering graph. An example is illustrated in Figure 1.

Figure I.



Comments

One might ask how accurate are these counting technics. The plate counting involves both plating error and dilution error. These errors are presented in Table V. In general the total inherent error of the plate counting technic may be as much as ±20 per cent. An example illustrating the variation in plate counts by surface streaking technic is presented in Table VI. The variation amounts to ±10 per cent. The counts of *Str. faecalis* obtained through three dilution-method for the most probable number (MPN) should be correct to first two significant figures (18). The computing formula for MPN is presented in Table VII. The inherent errors of all these technics presented are too minute to be considered when compared with any significant alteration in the fecal flora especially following antibiotic therapy.

TABLE VI
Precision of Plate Counts of Proteus (Surface—Streaking. Loh, 1952)

Set of Serial Dilutions	Dilution	Average No. of Colonies/Plate	Percentages
No. 1	10^{-7}	87	103
No. 2	10^{-7}	99	110
No. 3	10^{-7}	82	91
No. 4	10^{-7}	92	102
	Mean	90	100

Remarks: SS plates and 0.2 ml. for surface streaking were used. All the series of dilutions were prepared from the same 10^{-1} fecal suspension in water. The plates were inoculated in duplicate and were incubated at 37° C. for 48 hours before counting.

TABLE VII
Computing formula for the most probable number (MPN):

$$ay = [(1 - e^{-N_1\lambda})p]\ [(1 - e^{-N_2\lambda})r][(1 - e^{-N_3\lambda})t]$$
$$(MPN) \times [(e^{-N_1\lambda})q][(e^{-N_2\lambda})s][(e^{-N_3\lambda})u]$$

a = constant
N_1, N_2, N_3 = sizes of portions examined in cubic centimeters, such as 10 ml., 1 ml., 0.1 ml.
p, r, t = the numbers of potions of respective sizes giving positive tests for *Str. fecalis*
q, s, u = the numbers of negative tubes
λ = concentration of bacteria or trial number/cc.
e = base of Naperion log = 2.7118 - - - - - -

Discrepant results may arise from difference in technic. The writer is in entire agreement with Metzger and his associates (19) that discrepant results may be obtained with a simplified streak plate technic as compared with the serial dilution-pour plate technic. For better accuracy of the results the latter technic is highly recommended in this presentation.

Summary

A method for qualitative and quantitative studies of fecal flora is presented and discussed in detail. Serial dilution-pour plate or surface plate is the principal technic in this method. Three dilution-technic for the most probable number is used for enumerating *Streptococcus faecalis*. The total inherent error of these technics should not exceed ±20 per cent. This figure is too minute to be significant when compared with any significant alternation in the fecal flora especially following antibiotic therapy. The choice of medium for each group of the fecal flora is discussed wherever indicated.

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ABSTRACTS

THE ISOLATION OF HISTOPLASMA CAPSULATUM FROM SPUTUM AS A DIAGNOSTIC PROCEDURE

Gentry W. Moffit, et al, (Tennessee Department of Health), J. Lab. Clin. Med. 47, 499-502 (1956).

Histoplasma Capsulatum was isolated from twelve or 6.2% of 193 sputum specimens studied by the mouse inoculation method and in 4 or 2.1% by the direct culture method. The concentrated sputum specimens were inoculated by intraperitoneal injection. The mouse was sacrificed at the end of 28 days routinely. The spleen and liver were minced aseptically and inoculated onto three Sabouraud's glucose agar slants, one of which contained 20 units of penicillin and 40 units of streptomycin per milliliter. All cultures were incubated at 25° C. for 4 weeks with weekly examination.

STUDIES ON ANALOGUES OF L-CYSTEINE AND L-CYSTINE. I. SOME STRUCTURAL REQUIREMENTS FOR INHIBITING THE INCORPORATION OF RADIOACTIVE L-CYSTINE BY LEUKEMIC LEUKOCYTES

Austin S. Weisberger et al, (Western Reserve University, Cleveland), Blood XI, (1956).

The metabolism of leukocytes was studied by the role of radioactive sulfhydryl amino acid L-cysteine (L-cystine) in that process. The severe leukopenia induced by nitrogen mustard as modified by L-cysteine shows that its protective effect is distinctive. A depletion of L-cysteine in the diet of animals causes leukopenia, lack of cysteine in animals reduces the incidence of leukemia and addition of cystine increases incidence. Leukemic leukocytes were exposed to various analogues of cysteine (cystine) before incubation with S^{35} L-cystine. Highly specific structural and spatial configuration was found to be required to decrease the influx of S^{35} L-cystine.

In vitro selenium compounds (unlabeled) such as selenium cystine were effective. Their inhibitory effect may be due to competitive combination with specific intracellular receptors for L-cysteine (L-cystine), inactivation of enzymes or compounds essential for normal cellular function, alteration in membrane permeability or the toxic effect of selenium. These compounds may have important effects on leukemic leukocytes in vivo.

II. THE EFFECT OF SELENIUM CYSTINE ON MURPHY LYMPHOSARCOMA TUMOR CELLS IN THE RAT

Austin S. Weisberger and Leif G. Suhrland, (Western Reserve University, Cleveland), Blood XI, (1956).

Selenium cystine (diseleno-dialanine) which has a structure identical with cystine with the substitution of selenium for sulfur in the molecule, has been shown in vitro to decrease the incorporation of radioactive L-cystine by leukemic leukocytes. The effect both in vitro and in vivo was studied to determine a similar reaction in Murphy lymphosarcoma cells of a rat. The growth of the tumor was decreased as well as was the radioactive L-cystine decreased in incorporation by selenium cystine into the rat Murphy lymphosarcoma tumor cells both in vitro and in vivo. This decreased incorporation of L-cystine was associated with a decrease in the tumor in the animal. Since similar concentrations of selenium cystine decreased the influx of S^{35} L-cystine into human leukemic leukocytes, it is possible that selenium cystine may have a similar effect in leukemic leukocytes in vivo. These findings demonstrate with the decrease in tumor growth the possible importance of the SH amino acids and related SH compounds in the metabolism of malignant cells.

III. THE EFFECT OF SELENIUM CYSTINE ON LEUKEMIA

Austin S. Weisberger and Leif G. Suhrland, (Western Reserve University, Cleveland).

Patients with acute and chronic leukemia were studied by use of small amounts of oral selenium cystine. A rapid and striking drop in the total leukocyte count took place in all patients, the spleen decreased in size. Nausea and vomiting was so severe with the oral administration that it was not possible to administer this chemical long enough to determine whether an appreciable remission can be obtained in leukemia. Further study is necessary but so far these striking effects of an analogue of cystine on leukemia further suggest the importance of cystine (cysteine) in the metabolism of leukocytes.

CAPILLARIA HEPATICA

George M. Ewing and I. L. Tilden, (Straub Clinic, Honolulu), J. Pediat. 48, 341-8 (1956).

This study constitutes a fourth case report of true human infestation with Capillaria hepatica of the family Trichuridae which is a parasite of rodents although sometimes found in rabbits, dogs, pigs, monkeys and other animals but rare in humans. The life cycle of Capillaria hepatica is not completely understood. It is thought that the ingested embryonated eggs hatch in the intestine and reach the liver through the portal blood stream where the worms mature in three or four weeks. The eggs released in the liver remain there undeveloped until the death of the host.

The patient of this study was a fifteen month old female Caucasian known to eat dirt. Her family had a sick kitten and also kept dogs. The diagnosis was made from a stool specimen which showed large numbers of ova resembling those of Ascaris and later by a liver biopsy. The patient survived about a year from the onset of the disease.

A SIMPLE COLORIMETRIC METHOD TO DETERMINE SUGAR IN URINE

Francisco Moya and Joan Dewar, (Victoria General Hospital, Halifax, Nova Scotia), Jour. Lab. Clin. Med. 47, 314-19 (1956).

A simple quantitative procedure to determine urinary sugar by treating urine with Benedict's qualitative reagent is presented. The method uses Folin-Wu sugar tubes and measurements were made on the Coleman Junior spectrophotometer. The specimen of urine and Benedict's qualitative reagent are boiled in a Folin-Wu tube. Cooled and diluted to the 25 ml. mark with distilled water. If any precipitate is formed it is removed by filtration through Whatman No. 42 filter paper. The intensity of the blue color is measured at 650 mu. The color is stable at least two hours. There is a linear increase as the concentration of the glucose increases. The average difference between this procedure and Nelson's method is 0.20 grams per cent. This method also helps to rule out the false positives that the inexperienced personnel obtain with Benedict's qualitative reagent.

A HEMOLYSIN TEST FOR SELECTION OF UNIVERSAL DONORS

Dolores A. McDermott and Louis H. Muschel, (United Nations Blood Bank, APO 500, San Francisco), Amer. Jour. Clin. Path. 26, 4-12 (1956).

The presence of anti-A or anti-B isoantibodies of the so-called immune type has been incriminated as the cause of reactions resulting from the use of universal donor blood. Immune type antibodies possess various properties. They are characterized by their ability to act as hemolysins and fix complement. In the determination of isoimmunization caused by A B O incompatibility during pregnancy, the hemolysin test has been suggested as the best screening procedure.

The test described in this paper is designed to detect and measure isoantibodies capable of effecting red cell lysis in conjunction with complement. The authors report the results obtained in 1982 random group O serums.

0.1 ml. of donor's serum was pipetted into each of 3 small test tubes (12 x 75 mm). In a test for respective hemolysins, the first tube received 0.1 ml. of group A_1 red cells, and the second tube 0.1 ml. of group B red cells. The third tube was a complement control and is required because hemolytic activity depends on the combined action of hemolysin and complement. It contained 0.1 ml. of an inactivated positive reference serum and 0.1 ml. of the corresponding red cells in addition to the test serum. For a valid test, hemolysis must occur in the third tube. Incubate at 37° C for 1 hour and the extent of hemolysis was graded by visual estimate. Strongly hemolytic were regarded as potentially dangerous for universal use. The test is simple and requires no expensive equipment or reagents. The authors obtained a high degree of reproducibility in the described test procedure.

THE OCCURRENCE OF CONVULSIONS IN CHILDREN WITH SHIGELLA GASTROENTERITIS

William D. Donald, et al, (Medical College of Alabama), J. Pediat. 48, 323-7 (1956).

A series of 164 infants and children with diarrhea or dysentery is reported. The bacteriologic diagnosis of Shigella infection was made in 64 (39%); of these Sh. flexneri was found in 56 patients and Sh. sonnei from eight patients. Twenty more, or 45% of those infected with Shigella had convulsions.

It is noted that previously neurological manifestations were more frequently associated with Shigella dysenteriae (Shiga) than by other members of the Shigella group and that it has been accepted generally that this organism produces an exotoxin (neurotoxin). Clinically, the high incidence of convulsions in the group of patients in this study suggests the possibility that there may be a neurotoxin associated with the flexneri and sonnei organisms.

Bacteriologic studies were begun by obtaining a rectal swab from each patient shortly after admission before chemotherapy or antibiotics were started. Cultures were made immediately by inoculating S-S (Shigella-Salmonella) Agar and MacConkey Agar, the plates examined after twelve to twenty-four hours incubation and suspicious colonies transferred to Kligler Iron Agar. Those colonies on Kligler which showed typical Shigellae reactions were tested with Shigella grouping antiserum (Lederle) using the macroscopic slide technique. Biochemical reactions also confirmed the genus of Shigella production of acid only in dextrose, failure to ferment salicin or utilize citrate, methyl red positive and Voges-Proskauer negative. Sonnei group were slow lactose fermentators.

KEROSENE POISONING IN CHILDREN

Wm. D. McNally, (Spring Hill, Alabama), J. Pediat. 48, 296-9 (1956).

The accidental poisoning of 204 children by kerosene ingestion are reviewed with the purpose of examining the therapy and to instigate the labeling of kerosene and similar poisons as poisons.

The laboratory findings in these cases are given. Albumin varying from a trace to three plus was found in the urine of 30% of the cases. Reducing substances were found in six specimens, three had acetone. Red and white cells were found microscopically in several specimens. Eighty-five per cent had blood counts, of these seventy-three per cent had white cell counts over 10,000, those not developing pneumonia were from 7,850 to 19,500. Lymphocytes ranged from 10 to 79 per cent. Hemoglobin varied from 7 to 11.5 grams.

LISTERIA MENINGITIS

Peter L. Mathieu, Jr., et al, (Rhode Island Hospital, Providence), J. Pediat. 48, 349-55 (1956).

Meningitis due to Listeria monocytogenes in two cases are reported. It is pointed out that meningitis due to this organism simulates tuberculous meningitis in several respects. The spinal fluid may be clear. Both cases showed a pleocytosis with polymorphonuclear leukocytes predominating early and then lymphocytes predominating later. The organism was seen readily in the sedimented spinal fluid as a gram-positive rod about 0.5 by 1 to 2 microns in size (some larger were seen) and some showed a tendency to curve and were comma-shaped particularly after artificial cultivation. No branching, no spore-formation and no acid-fast characteristics were found. The organism grew well on ordinary culture media after incubation overnight. Colonies were small, round and measuring about 1-2 mm in diameter and resembled beta streptococci since they form a definite zone of beta hemolysis which is marked after 48 hrs. It ferments glucose, sucrose, and salicin with acid formation but no gas. Maltose and lactose show slow acid formation, mannitol showed no reaction. Similar gram-positive rod bacteria are eliminated by growth in 6.5% sodium chloride broth and growth at 4° C.

AMONG THE NEW BOOKS

COLLAGEN DISEASES: by John H. Talbott, M.D., Professor of Medicine, The University of Buffalo School of Medicine, Physician-in-Chief, Buffalo General Hospital, Buffalo, N. Y., and R. Moleres Ferrandis, M.D., Training Fellow in Arthritis of the Western N. Y. Chapter of the Arthritis and Rheumatism Foundation, 1956. Grune & Stratton, New York and London, 232 pages, 30 illustrations, 16 color plates. $6.50.

For each of the collagen disorders described there is a brief history, note on incidence and relation to other callogen diseases, etiology and pathogenesis as well as pathology and clinical findings, diagnosis, clinical course and treatment. Of definite interest to the technologist is each section on laboratory findings. The bibliography of 540 references is most complete. Systemic Lupus Erythematosus, Polyarteritis, Dermalamyositis, and Thrombotic Thrombocytopenic Purpura are the disorders that are discussed.

While no laboratory techniques are given, the discussions are of considerable value in relation to the over all picture.